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Jordan Journal of Pharmaceutical Sciences

Aims & Scopes:

***Jordan Journal of Pharmaceutical Sciences* (*JJPS*)** is a bimonthly open-access peer reviewed journal funded by the Scientific Research Fund at Ministry of Higher Education and Research and hosted by the Deanship of Research at the University of Jordan. JJPS is dedicated to various disciplines of pharmaceutical and allied sciences. JJPS publishes manuscripts on original work, either experimental or theoretical in the following areas:

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INTRODUCTION

The Jordan Journal of Pharmaceutical Sciences (**JJPS**) is a peer-reviewed Journal, which publishes original research work that contributes significantly to further the scientific knowledge in pharmaceutical sciences' fields including pharmaceutical/medicinal chemistry, drug design and microbiology, biotechnology and industrial pharmacy, instrumental analysis, phytochemistry, biopharmaceutics and Pharmacokinetics, clinical pharmacy and pharmaceutical care, pharmacogenomics, bioinformatics, and also **JJPS** is welcoming submissions in pharmaceutical business domain such as pharmacoeconomics, pharmaceutical marketing, and management. Intellectual property rights for pharmaceuticals, regulations and legislations are also interesting topics welcomed from our colleagues in Schools of Law.

On a current topic in Pharmaceutical Sciences are also considered for publication by the Journal. **JJPS** is indexed in SCOPUS (Q3). It's a journal that publishes 4 issues per year since 2021 in (**March, June, September, December**). The Editorial Team wishes to thank all colleagues who have submitted their work to JJPS). If you have any comments or constructive criticism, please do not hesitate to contact us at jjps@ju.edu.jo. We hope that your comments will help us to constantly develop **JJPS** as it would be appealing to all our readers.

Prof Ibrahim Alabbadi
Editor-in-Chief
School of Pharmacy- The University of Jordan
Amman 11942- Jordan

Volume 16, 2023

Letter from the Editor-in-Chief

After a full year of getting back to normal life in 2022, with all work including editorial board meetings performed face to face, the Jordan Journal of Pharmaceutical Sciences (JJPS) will continue to publish 4 issues annually at regular times i.e., quarterly, but the good news that each issue every quarter will have 15 accepted articles to be published per issue (instead of 10). The latter indicates the good achievement of JJPS last year as much more submissions were received from international countries representing 70% of total submissions while 30% were received from Jordan. Furthermore, this will decrease waiting times for researchers in receiving decisions regarding whether their submissions are either accepted or not. Also increasing the number of articles published per issue will again increase researchers' satisfaction and not delay publishing their accepted work, for example, the waiting time from receiving the submission through the decision to publishing decreased from 34 weeks in (2019-2020) to 22 weeks in (2021-2022) on average.

On the other hand, the number of citations exceeded 2 folds of the number of articles published looking forward to reaching the Q2 category in SCOPUS soon; thanks to all colleagues on the editorial board, local as well as international advisory board scientists, also special thanks to all researchers for their belief and trust in JJPS.

One important issue worth mentioning this year is the challenge of using Artificial Intelligence in writing scientific papers using new applications such as Chat GPT which since launched last November was spread not only very fast but in acceleration way all over the world. We are observing and will try to meet with all stakeholders in our field very soon to have deep discussions hoping to reach a solution to such a threat mainly in similarity percentages reports for the submissions.

In JJPS, we will continue encouraging researchers to submit their original research as well as systematic reviews and commentaries emphasizing our commitment to complete reviewing the submissions by a group of excellent scholars in a scientific logical transparent way in a short time.

Best regards

Prof Ibrahim Alabbadi
Editor-in-Chief

Editorial Commentary

Dear colleagues and researchers

Allow me to introduce myself. I am Prof. Dr. Udo Bakowsky from Philipps Universität Marburg, Germany. My research interests encompass Pharmaceutics, Nanotechnology, Drug Delivery, Photodynamic Therapy, and Gene Therapy.

The role of the Editorial Advisory Board is to provide input, suggestions, and specialized scientific support in journal management. We may also act occasionally as Guest Associate Editors for specific papers or Guest Editors for special thematic issues, propose opinion papers, editorials, or reviews, and serve as reviewers for problematic or controversial papers. In conjunction with other members of the Editorial Board, the Editorial Advisory Board will act as ambassadors for our journal within the technical and scientific community.

As a tenured professor and Head of the Department of Pharmaceutics and Biopharmaceutics at the School of Pharmacy, University of Marburg, Germany, I currently lead a group of more than 50 researchers (currently, 35 PhD students). With over 25 years of experience in drug delivery and therapeutic biomaterials, including applications such as antibacterial coatings, stents, gene therapy, chemotherapy, and photodynamic therapy, I have authored more than 250 peer-reviewed articles, several book chapters, and have filed over a dozen patents. Additionally, I have co-authored approximately 750 presentations at various international meetings. Google Scholar indicates that my work has been cited over 13,000 times, with an h-index of 65.

(https://scholar.google.de/citations?view_op=list_works&hl=de&hl=de&user=KdQV-rwAAAAJ&pagesize=80) .

My laboratory primarily focuses on research and teaching related to conventional and modern pharmaceutical dosage forms, with an enrollment of 165 students per semester. My team of researchers is dedicated to various topics, including modern nanoscale dosage forms. We aim to enhance the effectiveness and compliance of poorly soluble drugs while addressing critical issues such as drug-resistant infections and tumors. Our primary areas of interest encompass colloidal drug delivery systems, biodegradable polymer nanoparticles, and liposomes. We also delve into diagnostics involving MRI and ultrasound, molecular recognition, ligand-receptor interactions, lipids, lipid model membranes, as well as their interactions with proteins or DNA. Additionally, we explore surface modification of biomaterials and implants, along with the formulation of DNA/lipid complexes. Our major application areas include tumor therapy (pancreatic, lung, ovarian, angiomas, etc.), arteriosclerosis (ultrasound diagnostics and therapy), and coronary inflammation. We hold a special interest in the application of scanning probe/atomic force microscopy techniques on biological systems.

I am dedicated to promoting the growth and development of all my Ph.D. students, aiming to achieve both scientific excellence and complementary skills that I consider crucial for their future careers. It is mandatory for my Ph.D. students to enroll in a graduate school to participate in courses that enhance their specialized complementary skills. We begin guiding them as early as possible in writing their first research papers, book chapters, and review papers, and we involve them in the peer-review process of research papers to which I am invited.

Our Ph.D. students receive thorough monitoring and are each assigned a postdoctoral supervisor for the duration of their Ph.D. One of the most important tasks of our research team as mentors during this stage is to utilize our networks and knowledge to support the postdocs/fellows in advancing their careers. This includes identifying the most suitable funding schemes for further funding or employment opportunities.

I am delighted to serve as a member of the advisory editorial board of the Jordan Journal of Pharmacy Sciences. It is my pleasure to extend an invitation to all researchers to contribute their scientific work to JJPS. We welcome not only papers that align with the journal's scope but also those that may fall outside it or might not be groundbreaking but are relevant in the context of legislation and are of interest to pharmacy readers.

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CONTENTS

Jordan Journal of Pharmaceutical Sciences, Volume 16, No. 3, 2023

Intraoperative Insulin Infusion Regimen versus Insulin Bolus Regimen for Glucose Management during CABG Surgery: A Randomized Clinical Trial

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ABSTRACT

Background and Aim: The stress induced by surgery disrupts the delicate balance between hepatic glucose production and glucose utilization in the body. Despite the significance of intraoperative glycaemic control for diabetic patients, limited attention has been given to this aspect. Two methods for administering insulin to manage glucose levels during surgery exist. This study aimed to compare intraoperative glucose levels in diabetic patients undergoing Coronary Artery Bypass Graft (CABG) surgery using either insulin infusion or the bolus method.

Method: This was a Randomized Clinical Trial (RCT). Seventy diabetic patients aged 40 or older scheduled for CABG surgery were enrolled in the trial. They were randomly assigned, using block randomization, to receive intraoperative insulin via either infusion or the bolus method. The primary outcome measure was intraoperative glucose levels. Subsequent insulin unit requirements and intraoperative potassium levels were secondary outcomes. Data was monitored throughout the CABG procedure and recorded at six different checkpoints.

Results: Male patients constituted the majority in both groups, with no significant differences in the preoperative characteristics of patients, including HbA1c levels and comorbidities. The infusion regimen demonstrated a statistically significant reduction in glucose levels (-19.12 mg/dL, 95% CI: -27.68 to -10.55, P<0.001, Cohen's d=1.06) compared to the bolus regimen. The total insulin units administered in the infusion group were 480 units, as opposed to 600 units in the bolus group (P=0.001, Cohen's d=0.85). Importantly, no cases of hypoglycemia or hyperkalemia were reported among the patients.

Conclusion: Intraoperative glucose control using insulin was effective for CABG patients with diabetes. However, the infusion regimen exhibited statistically superior results compared to the bolus regimen.

Clinical Trials Registry and Registration Number: The trial received approval from the Ethics Committee on 2/1/2019/2020 and was registered on Clinicaltrials.gov under ID: NCT04824586.

Keywords: Type 2 diabetes mellitus; cardiac surgery; glucose levels; insulin infusion; insulin bolus.

HIGHLIGHTS:

- Most studies typically focus on pre- and post-operative glucose levels. This is the first Randomized Clinical Trial to compare intraoperative glucose level performance between infusion and bolus regimens.
- The use of insulin for glucose control in CABG patients helps prevent intraoperative hyperglycemia and potassium disturbances.
- The insulin infusion regimen in diabetic patients during CABG surgery yielded superior outcomes compared to the bolus regimen.

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INTRODUCTION

In cardiac surgery patients, a significant association between intraoperative hyperglycaemia (glucose greater than 200 to 250 mg/dL) and increasing odds of morbidity and mortality has been documented1,2. At such high glucose levels, there are increased risks of pulmonary and renal complications, infection, atrial fibrillation, heart failure, myocardial infarction, pericarditis, and neurological complications[3-7].

Nevertheless, the optimal glucose management strategy during operations remains undetermined[8,9]. There is some evidence supporting the superiority or at least equivalence of moderate glycaemic control (100–140 mg/dL, or 140–180 mg/dL) compared to intensive control (80–110 mg/dL) in patients undergoing cardiac surgery[10,11]. It was found that glucose levels ranging from 140-170 mg/dL had the lowest risk of adverse outcomes[10]. The established and widely approved treatment strategies in cardiac surgery are still predominantly insulin-based[12-14]. Nevertheless, to the best of our knowledge, there is no universal agreement on the best specific protocol to be used. A considerable body of literature compares the impact of infusion insulin versus the sliding scale on postoperative parameters including surgical site infections and readmission rates[14-15]. Despite that, no published research, including a review of existing literature from sources such as PubMed, CINAHL, EMBASE, and CENTRAL, has compared the practical aspects and intraoperative parameters between infusion insulin and bolus insulin protocols in cardiac surgery for patients with diabetes. Reviewers, such as Duggan and colleagues, have confirmed the lack of data comparing subcutaneous insulin to IV insulin infusion in the operative setting[15-17].

Accordingly, the primary objective of the present trial was to explore which insulin-based regimen, either infusion or bolus regimen, is superior for intraoperative management of glucose levels in patients with diabetes undergoing Coronary Artery Bypass Graft (CABG) surgery. Secondary objectives include comparing the relative amounts of insulin required during the operation, the subsequent cost impact, and comparing potassium levels between the two groups.

METHODS AND MATERIALS

Study Design

This study was a parallel group, randomized clinical trial (RCT) with a 1:1 allocation ratio. The study was designed and reported in accordance with CONSORT guidelines.

Ethical Approval and Study Registration

Ethical approval for the study was obtained from the Office for Research Ethics Committees at Hashemite University and Prince Hamza Hospital in Jordan, with reference number 2/1/2019/2020. The study was also registered on Clinicaltials.gov (ID: NCT04824586)[18].

Participants

The eligibility criteria for participants were adult patients with type 2 diabetes mellitus admitted to the hospital for CABG surgery. These patients were asked to provide informed consent and met the following criteria: ages ranging from 40 to 70 years, a regular need for insulin according to dosing guidelines, and preoperative glucose levels between 200 mg/dL and 300 mg/dL.

The following patients were excluded from the trial: insulin-sensitive patients[19], insulin resistance patients (Body Mass Index (BMI) > 35 kg/m^2, total daily insulin dose > 80 units, and/or daily steroids therapy > 20 mg prednisone), age > 70 years, Glomerular Filtration Rate (GFR) < 45 ml/min, individuals with no history of diabetes, patients at high risk of complications, and those whose operations were to be supervised by a specialized team. Patients unable to provide written informed consent and those with ≥ 4 emergency admissions within the six months prior to the index admission were also excluded.

Setting

Patients were recruited from the tertiary care center at Prince Hamza Hospital in Amman, Jordan. Patients with diabetes who had scheduled cardiac surgery and met the

study criteria were invited to participate. Patients who accepted participation and provided their consent were enrolled by well-trained research assistants who were trained in ethical standards and a patient-centered approach.

The Intervention

All patients in the two groups, the infusion and bolus groups, received doses of fast-acting human insulin (Regular insulin, Actrapid®). The insulin regimen protocol and its details were executed following the insulin standardization protocol in the hospital[17].

Primary and Secondary Outcomes

The primary outcome was the intraoperative glucose level. It was monitored six times during the operation (see Table 1). The checkpoints were as follows: induction measurement before surgery, glucose levels post-heparin, and then every 30 minutes for two hours while the patient's blood was circulated through the Coronary Artery Bypass Machine (CABM). Insulin doses and potassium levels were recorded for use in the analysis of secondary outcomes.

Table 1 Baseline demographic and clinical characteristics of patients for two groups (n=70)

Variables	Infusion Group (n=35)	Bolus Group (n=35)	P-Value
Age (years) Mean (±SD)	57.6 (±6.98)	58.3 (±7.52)	0.69[a]
Gender: Male (%)	28 (80.00%)	29 (82.86%)	0.76[b]
Duration of diabetes (years) Mean (±SD)	10.06 (±7.80)	7.06 (±6.90)	0.09 [a]
HbA1c % Mean (±SD)	9.7 (±2.37)	9.2 (±2.88)	0.40 [a]
Induction glucose level mg/dL Mean (±SD)	244.77 (±26.56)	241.29 (±23.65)	0.56 [a]
No. of patients on insulin preoperatively (%)	9 (25.71%)	4 (11.43%)	0.12 [b]
No. of patients on oral hypoglycaemic agents (%)	24 (68.57%)	29 (82.82%)	0.16 [b]
No. of patients on both insulin and hypoglycaemic agents (%)	2 (5.71%)	2 (5.71%)	1.00 [b]
Hypertension (%)	22 (62.86%)	27 (77.14%)	0.19 [b]
Duration of Hypertension (years) Mean (±SD)	9.7 (±7.43)	11.8 (±7.58)	0.35 [a]
Duration of ischemic heart disease (weeks) Mean (±SD)	25 (±8)	31 (±5)	0.07 [b]
Duration of ischemic heart disease (years) Mean (±SD)	1.3 (±1.58)	1.1 (±1.46)	0.73 [a]
Kidney Disease (%)	2 (5.71%)	1 (2.86%)	1.00 [c]
Thyroid Disease (%)	1 (2.86%)	0	1.00[c]

Sample Size

To detect a difference of at least 25 mg/dL between the infusion and bolus groups (the standard deviation of the two groups is expected to be 35 mg/dL, i.e., the variance is 1225 mg/dL), the study recruited and collected complete data for a minimum of 31 patients in each group. This provided a confidence level of (95%) and the power of 80%. $n = (Z\alpha/2+Z\beta)^2 * 2*\sigma^2 / d^2$. where $Z\alpha/2$ is the critical value of the Normal distribution at $\alpha/2$ (for a confidence level of 95%, α is 0.05, and the critical value is 1.96), $Z\beta$ is the critical value of the Normal distribution at β (for a power of 80%, β is 0.2, and the critical value is 0.84), σ^2 is the population variance, and d is the difference needed to be detected[20].

Statistical Methods

Standard independent-samples t-tests or separate variances t-tests (Welch t-tests) were used to compare the results between the two arms of the study. A General Linear Model and one-way repeated measures ANOVA were conducted to determine whether there was a statistically significant difference within groups.

Cost analysis and cost-effectiveness were employed for the pharmacoeconomic analysis[21,22]. The incremental cost-effectiveness ratio (ICER) was calculated using the following equation:

$$\text{ICER} = (\text{Cost of insulin in the infusion protocol} - \text{Cost of insulin in the bolus protocol}) / (\text{Drop in glucose level by infusion} - \text{Drop in glucose level by bolus})$$

Equation 1

Randomization, Allocation and Blinding

During patient enrollment, concealed allocation to either the infusion group or bolus group was ensured by using a closed envelope system prepared by an independent investigator[23]. Block randomization with random block sizes was employed to ensure allocation balance and prevent selection bias by avoiding allocation prediction[24]. Researchers and physicians were blinded to the block size sequence and randomization. The envelopes remained unopened until the registration of patients was completed. Hospital staff responsible for monitoring glucose levels and administering insulin were also blinded to the primary and secondary outcomes of the study.

RESULTS

Out of 179 screened patients, 93 patients were invited to participate in the study, and ultimately, 70 patients were recruited and randomized into two arms. No losses or exclusions were documented after recruitment in the study. Please refer to Figure 1 below for the flow of participants through the trial based on eligibility criteria. The patient recruitment process took place from June 1, 2019, to January 30, 2020. Follow-up was not carried out as the study's focus was on intraoperative glucose control.

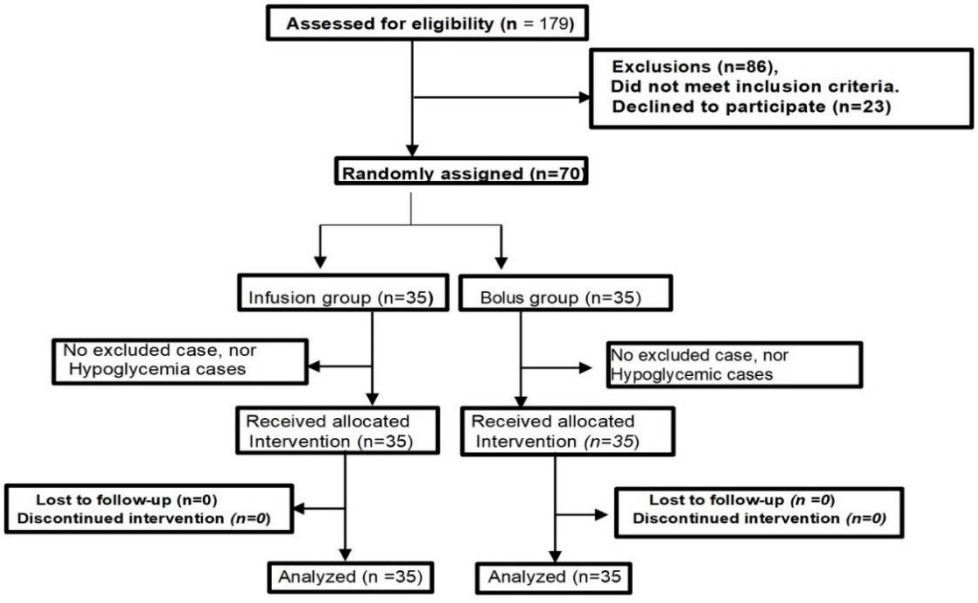

Figure 1 Participation flow diagram. Patients recruited and randomized (n=70); in the infusion group (n=35) and bolus group (n=35)

Characteristics of Study Participants

The baseline characteristics of the 70 subjects presented in Table 1, with no statistically significant differences were noted in all characteristics of the two groups: infusion group (n=35) and bolus group (n=35). There were more male participants than female participants in the two groups with an average age 57.6 and 58.3 years old, respectively. Mean HbA1c for the two groups were between 9.7 and 9.2, respectively. Induction glucose levels were much closed with 244.77 and 241.29 mg/dL. In addition, there were no significant differences in the presentation of other chronic diseases.

Surgery Characteristics

The number of diseased vessels (blocked coronary arteries) in patients was 3 in 91.4% and 94.3% in the infusion group and the bolus group, respectively. The remaining patients had 4 diseased vessels. The number of grafts performed was equal to the number of diseased vessels in all patients in the two groups. There were no significant differences in the number of diseased vessels and grafts performed between the two groups.

The average surgery time for patients with three grafts in both groups was 5.0 ± 0.4 hours, and 5.3 ± 0.5 hours for patients with four grafts. There were no significant differences in operative time between the two groups.

A team consisting of surgeons, anesthesiologist, and surgical nurses conducted the CABG surgeries for all patients included in this trial.

Pre- and Intraoperatively Glucose Levels

Table 2 displays the mean glucose levels between the two insulin groups. Variances were found to be homogeneous, as assessed by Levene's test for equality of variances. Glucose levels across the operation were statistically significantly lower in the infusion group compared to the bolus group. The maximum difference was observed at the second checkpoint, where the mean difference was -24.91 mg/dL (95%CI: -40.73 to -9.10; P=0.002) with a medium effect size (Cohen's d value = 0.75). The minimum statistically significant difference was reported at checkpoint 5, where the difference was –17.6 mg/dL (95% CI: -29.07 to -6.13; P=0.003, d=0.73). At the end of operation mean glucose levels for the infusion group was 19.12 mg/dL less than mean for the bolus group (95% CI: -27.6 8 to -10.55, P<0.001, d=1.06).

Table 2 Primary outcome, glucose level as measured at six checkpoints through the operation and insulin units used in the CABG operations (n=70)

Checkpoints	Variables between-group analysis	Mean glucose level (±SD) (mg/dL) Infusion Group (n=35)	Mean glucose level (±SD) (mg/dL) Bolus Group (n=35)	Levene's Test for Equality of variances	Mean difference	P value	95% Confidence Interval of the difference		Effect size Cohen's d
							Lower	Upper	
1	Induction Glucose level	244.77 (±26.56)	241.29 (±23.65)	0.326 [a]	3.48	0.564 [b]	-8.51	15.45	0.14
2	Glucose / Post Heparin	193.06 (±33.71)	217.97 (±32.59)	0.243 [a]	-24.91	**0.002** [b]	-40.73	-9.10	0.75
3	1st on CABM	169.46 (±31.55)	191.49 (±30.88)	0.972 [a]	-22.03	**0.004** [b]	-36.92	-7.13	0.71
4	2nd on CABM	158.00 (±25.42)	176.11 (±28.71)	0.398 [a]	-18.11	**0.007** [b]	-31.05	-5.18	0.67
5	3rd on CABM	156.71 (±25.37)	174.31 (±22.66)	0.661 [a]	-17.60	**0.003** [b]	-29.07	-6.13	0.73
6	4th on CABM post-protamine	152.37 (±19.31)	171.49 (±16.48)	0.540 [a]	-19.12	< **0.001** [b]	-27.68	-10.55	1.06
		Mean (±SD) Insluin Unit	Mean (±SD) Insluin Unit						
		480 [13.71 (±4.29)]	600 [17.14 (±3.80)]	0.354	-3.43	0.001	-5.36	-1.50	0.85

CABM: Coronary Artery Bypass Machine *a Population variance of both groups is equal* *b Standard independent-samples t-test*

No cases of hypoglycaemia (glucose level < 60 mg/dL) were reported during the trials in either of the two groups.

Within-group analysis

Figure 2A illustrates the decrease in glucose levels in the infusion group from 244.77 (±26.56) mg/dL pre-intervention to 152.37 (±19.31) mg/dL (i.e. reduction of 92.4 mg/dL, 95% CI: 75.7–109.1, p <0.001) and in the bolus group from 241.29 (±23.65) mg/dL to 171.49 (±16.48) mg/dL (i.e. reduction of 69.8: 95% CI: 56.9–82.7, p <0.001) by the end of intervention. Both the infusion and bolus interventions elicited statistically significant changes in glucose concentration over time, $F(2.9, 97.9) = 97.86$, $p < 0.001$ and $F(2.6, 89.4) = 75.07$, $p < 0.001$, respectively.

Mauchly's test of sphericity indicates that the assumption of sphericity has been violated in both the infusion group $\chi^2(14) = 58.34$, $p < 0.001$ and the bolus group $\chi^2(14) = 76.74$, $p < 0.001$, so results were interpreted by using the Greenhouse-Geisser correction (estimated epsilon (ε) less than 0.75). The sample effect size based on within-subjects factor variability, partial eta squared effect size η^2 was = 0.74 in the infusion group, and 0.67 in the bolus group. The estimated effect size (partial ω^2) was = 0.697 in the infusion group and 0.638 in the bolus group.

Post hoc pairwise analysis, adjusted for multiple comparisons Bonferroni correction, revealed that glucose concentration was statistically significantly decreased within the infusion group between pairwise at checkpoints 1, 2, and 3 ($p < 0.05$). A similar pattern has also resulted in post hoc pairwise analysis for the bolus group.

Insulin Units Used Pre- and During Surgical Operations

As shown in Figure 2B, patients in the infusion group received fewer insulin units compared to the bolus group, with 13.71 (±4.29) units and 17.14 (±3.80) units, respectively (Table 2). The difference was statistically significant (-3.43 units of insulin, 95% CI: -5.35 to -1.50, P = 0.001, d = 0.85).

Cost Effectiveness Analysis

Economic evaluation ICER = (480 unit * $0.15/unit – 600 unit * $0.15/unit) - (92.4 - 69.8). In other terms = 72-90 / 22.6 = -18/22.6 = -0.79. (See Equation 1).

As the incremental cost is negative (-18) and the incremental effect is positive (22.6), the infusion intervention is unequivocally cost-effective when compared with the bolus intervention. It is dominant, achieving better outcomes at a lower cost[21,22].

Potassium Levels

Generally, there was no case of hypokalaemia, and potassium levels reached the upper limit only once (at checkpoint 5) in the bolus infusion group. However, the results showed that there were no statistically significant differences between the two groups with regards to potassium levels throughout the operations. Within-group analysis revealed that the mean potassium levels fluctuated between 4.13 (±0.39) and 4.43 (±0.38) among the infusion group and 4.09 (±0.32) and 4.51 (±0.41) among the bolus group. Figure 3 illustrates the intraoperative levels of potassium across six operation checkpoints.

Both groups reported within-group statistically significant differences in potassium levels. Post hoc pairwise analysis, adjusted for multiple comparisons using Bonferroni correction, revealed that statistically significant results were driven by checkpoint 5 in both groups (see Figure 3). The maximum fluctuation and statistically significant mean difference in the infusion group were observed at 5, 1 Δ mean = 0.29 (95% CI: 0.06 – 0.53, P=0.006) and points 5,6: Δ mean = 0.30 (95% CI: 0.08 – 0.52, P=0.002). In the bolus group, the maximum mean difference was observed at points 5, 1: Δ mean = 0.42 (95% CI: 0.14 – 0.70, P=0.001) and points 5,6: Δ mean = 0.33 (95% CI: 0.004 – 0.66, P=0.045). The maximum mean difference in the bolus group was larger than that in the infusion group. However, all potassium readings intraoperatively were almost within the normal potassium range.

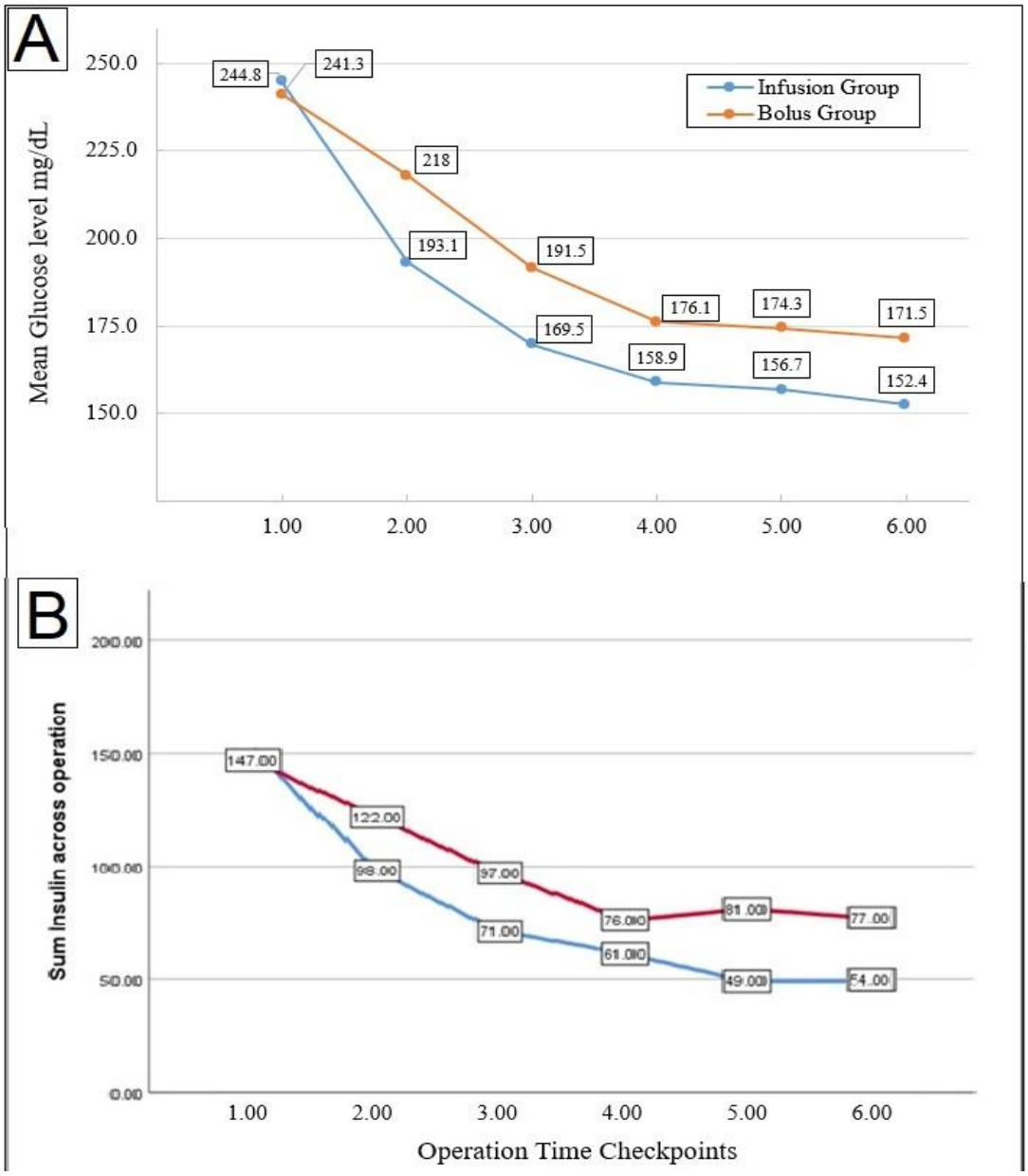

Figure 2A: Intraoperative mean glucose levels across six operation checkpoints infusion (n=35) or bolus (n=35) groups. By the end of the intervention, the decrease in glucose levels was significant within both the infusion group, p<0.001, and bolus group p<0.001 by one-way repeated measures ANOVA. 2B: Intraoperative insulin units used across six checkpoints operation. Patients within the infusion group received fewer total insulin units that those in bolus group. P=0.001 by the standard independent-samples t-test.

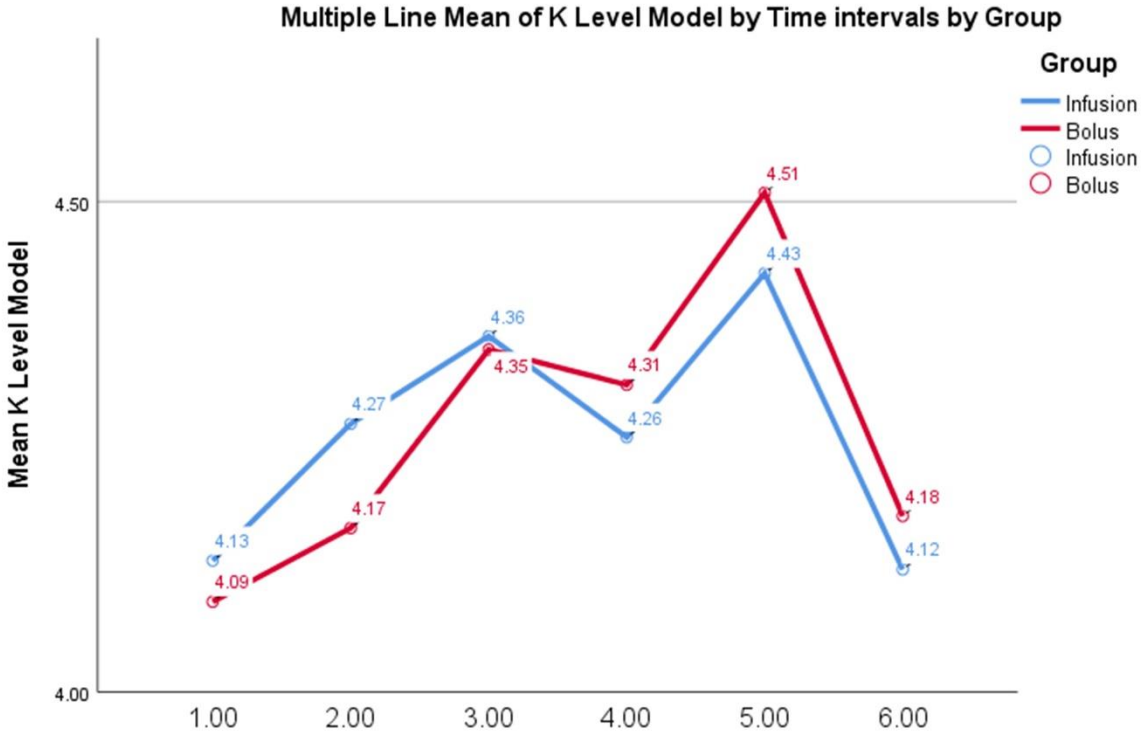

Figure 3 Intraoperative levels of potassium across six operation checkpoints. Patients received insulin as infusion (n=35) or bolus (n=35). By the end of the intervention, change in potassium levels was significant within both the infusion group, p<0.001, and bolus group p<0.001, one-way repeated measures ANOVA.

Post-operative data

As per the trial protocol, data regarding patients were collected in the Cardiac Intensive Care Unit (CICU) for six hours post-operatively, focusing on the general health status of the patients. Both groups exhibited controlled glucose and potassium levels, and no insulin-related complications were observed. Additionally, during these hours in the CICU, patients in both groups received a nearly identical amount of insulin as that administered during the last intraoperative checkpoint. Furthermore, patients from both groups remained stable at the sixth hour post-operatively.

DISCUSSION

This RCT included two groups with 1:1 allocation ratio. Seventy patients with diabetes who underwent This randomized controlled trial (RCT) featured two groups with a 1:1 allocation ratio, including seventy patients with diabetes who underwent CABG surgery. The patients in the infusion regimen group (n=35) demonstrated a statistically significant impact on blood glucose level reduction compared to the bolus group (n=35). Moreover, the infusion group received a lower total amount of insulin units than the bolus group. Notably, there were no reported cases of hypoglycaemia and hyperkalaemia in any of the patients receiving the two regimens.

This study represents the first evaluation, to the best of our knowledge, comparing bolus versus infusion methods in patients with diabetes undergoing cardiac (CABG) surgery. These findings align favorably with the joint French diabetology and anesthesiology position statement,

which recommended fewer insulin units with the infusion method compared to the bolus method, even though substantial evidence supporting such a recommendation was lacking[24].

The findings of the present study contrast with results in published research that compared the two insulin regimens in non-cardiac surgeries[25]. Arun et al. concluded that the intravenous insulin bolus regimen, compared to the insulin infusion regimen for intraoperative blood glucose management in non-cardiac surgery, provided better glycaemic control measured in terms of the proportion of intraoperative duration during which the patients remained within the target blood glucose levels[25]. This somewhat contradictory result may be attributed to differences in patient characteristics and variations in perioperative blood glucose levels.

Hyperglycemia commonly observed during cardiac surgery results from a combination of exogenous glucose administration, glucose utilization during prolonged anesthesia, and the relative insulin resistance that develops in response to the stress of surgery[26]. While there is strong evidence that preoperative and postoperative glucose control for patients undergoing cardiac surgery impacts surgical-related complications[27,28], there is limited data on methods of intraoperative insulin administration. Glucose management has been assessed in a few types of cardiac surgery using both bolus insulin and insulin infusion. In the Kruger et al. study, the use of a timely insulin dosing method in patients with diabetes during cardiopulmonary bypass (CPB) surgery was effective but raised some safety concerns in preventing hyperglycemia during surgery[26].

The findings related to insulin unit consumption align with the performance of blood glucose levels during heart surgery. The results regarding potassium levels were not unexpected. Albacker et al., in their study comparing 44 patients undergoing elective CABG, who received titrated intravenous insulin infusion (n = 22) or a fixed high-dose systemic insulin infusion (n = 22), did not find any differences in potassium levels between the two groups and did not observe any hypo- or hyperkalaemia during the study[13]. Despite differences in study design, this may provide insights into the results related to potassium level performance.

Limitation of the study

The present study has limitations. It was conducted at a single center, which is a constraint. Additionally, the study design had limitations, with a short follow-up after the operation. However, post-operative follow-up may relate to glucose levels or other variables rather than the method of insulin administration. Furthermore, the trial's inclusion criteria were limited to stable patients requiring regular insulin, making the results applicable primarily to patients within similar groups. The study did not explore perioperative treatment modalities, as inclusion criteria focused on glucose levels between 200 – 300 mg/dL. Finally, while a cost-effectiveness analysis of the cost of insulin was performed, no other cost factors were considered.

CONCLUSION

Using insulin in both infusion and bolus regimens intraoperatively in patients with type 2 diabetes mellitus undergoing CABG surgeries was effective in controlling glucose levels during the operation without influencing potassium levels. However, the present randomized clinical trial demonstrated that providing insulin through the infusion regimen delivers statistically significantly better intraoperative glucose control for patients with diabetes undergoing CABG surgery when compared to the bolus regimen. Consequently, the infusion regimen required fewer units of insulin and exhibited dominant cost-effectiveness, achieving better outcomes at a lower cost.

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DISCLOSURE OF ETHICAL STATEMENTS

- Research Protocol Approval: The research protocol was approved by the Office for Research Ethics Committees at Hashemite University and Prince Hamza Hospital, Jordan, with reference number 2/1/2019/2020.
- Informed Consent: All participants signed the consent form.
- Approval date of Registry and the Registration No. of the study/trial: The study was registered on Clinicaltials.gov (ID: NCT04824586).
- Animal Studies: N/A

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مقارنة تأثير طرق إعطاء الإنسولين ببطء أو بدفعة واحدة على إدارة مستويات الجلوكوز أثناء العمليات الجراحة لتوصيل شرايين القلب التاجية : دراسة سريرية عشوائية محكمة

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ملخص

الإطار العام والهدف: إجراء العمليات الجراحة يؤثر على التوازن المنظّم بين إنتاج الجلوكوز في الكبد واستخدامه في الجسم. على الرغم من أهمية مراقبة مستوى الجلوكوز أثناء العمليات الجراحية لمرضى السكري، إلا أن هناك قليل من الدراسات التي بحثت في هذا الأمر. هناك طريقتان لإعطاء الإنسولين للسيطرة على مستوى الجلوكوز في المرضى أثناء الجراحة (إما ببطء أو دفعة واحدة). هدفت هذه الدراسة إلى مقارنة مستويات الجلوكوز لمرضى السكري أثناء عمليات جراحة القلب لتوصيل الشرايين التاجية باستخدام الإنسولين ببطء أو بالجرعة الواحدة.

الطريقة: دراسة سريرية عشوائية محكمة. تم انتقاء سبعون مريضًا مصابًا بالسكري (بعمر أكثر من 40 عامًا) والذين كانوا في مواعيد لعملية جراحة القلب . تم تعيينهم باستخدام طريقة بحثية متوازنة لتلقي الإنسولين أثناء العملية إما ببطء أو بنظام الجرعة الواحدة. كان مؤشر النتائج الأساسي هو قياس مستوى الجلوكوز أثناء العملية. وكانت كمية وحدات الإنسولين المستخدمة ومستويات البوتاسيوم أثناء العملية مؤشرات نتائج ثانوية. تم مراقبة البيانات خلال العملية وسُجِّلت على مدى ست نقاط تقييمية أثناء العملية الجراحية.

النتائج: كانت نسبة المرضى الذكور أعلى في المجموعتين مع عدم وجود فروق ذات دلالة إحصائية في خصائص المرضى قبل العمليات. أظهرت الطريقة العلاجية بإعطاء الانسولين ببطء أنها أدّت الى انخفاضًا ذو دلالات إحصائية معتمدة في مستويات الجلوكوز مقارنة بذلك الانخفاض من خلال اعطاء الانسولين دفعة واحدة- (-19.12 mg/dL, 95% CI: -27.6 8 to -10.55, P<0.001, Cohen's d=1.06) . كانت كمية وحدات الإنسولين الإجمالية للمجموعة التي تم استعملت الانسولين ببطء 480 وحدة مقارنة بـ 600 وحدة للمجموعة التي أخذت الإنسولين بالجرعة الواحدة، و هذا الفارق كان ذو دلالة احصائية معتمدة (فرصة الاحتمالية أقل من واحد بالالف). وأخيرًا، لم تتم اية ابلاغات عن حدوث انخفاض في مستوى الجلوكوز أو ارتفاع مستوى البوتاسيوم في المرضى .

الخلاصة: كانت السيطرة على مستوى الجلوكوز أثناء عمليات القلب الجراحية باستخدام الإنسولين فعّالة لمرضى السكري. وأظهرت هذه الدراسة أن طريقة اعطاء الانسولين ببطء تعطي نتائج أفضل وبدلالة إحصائية معتمدة من طريقة اعطاء الانسولين بالدفعة الواحدة.

الكلمات الدالة: مرض السكري من النوع الثاني؛ جراحة القلب؛ مستويات الجلوكوز؛ طريقة إعطاء الإنسولين ببطء؛ طريقة اعطاء الإنسولين بالجرعة الواحد؛ إدارة مستويات الجلوكوز؛ إدارة مستويات الجلوكوز خلال العمليات الجراحية.

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Reverse Vaccinology Analysis of B-cell Epitope against Nipah Virus using Fusion Protein

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ABSTRACT

Nipah virus (NiV) is an RNA virus, a pathogenic paramyxovirus that causes nonlethal respiratory illness in pigs. It was originally reported in Malaysia in 1998. NiV is considered a potential outbreak threat because it is zoonotic. However, no vaccines or antiviral drugs have been found against NiV. Therefore, the main objective is to develop effective vaccines by characterizing the fusion protein of NiV. We used a reference sequence retrieved from the National Center for Biotechnology Information (NCBI), then 3D modeled it to obtain the conserved region of the fusion protein. The interaction between the conserved region and B-cell receptors has been evaluated through a molecular docking approach. The B-cell epitope was identified using the Immune Epitope Database (IEDB) web server. As a result, we recommend Pep_D FANCISVTCQCQ as an epitope-based peptide vaccine candidate against Nipah virus. Pep D is highly immunogenic and does not cause autoimmune reactions. Pep D has the lowest binding energy for BCR molecular complexes, which can activate the transduction signal and direct B-cell immune response. However, further studies are required for confirmation (in vitro and in vivo).

Keywords: Fusion protein, Nipah virus, reverse vaccinology, immunoinformatic.

INTRODUCTION

Nipah virus (NiV) is an RNA virus, a pathogenic paramyxovirus originally reported in Malaysia in 1998, primarily causing nonlethal respiratory illness in pigs [1]. NiV is highly pathogenic to a wide range of mammals due to its zoonotic transmission (from bats to humans, or from bats to pigs and then to humans) as well as human-to-human transmission [2]. Therefore, this virus has the potential to cause outbreaks.

Nipah virus belongs to the Paramyxoviridae family and is the second member of the Henipavirus genus. The virus is named after Kampung Sungai Nipah, a village in Negeri Sembilan where pig farmers were found to have encephalitis [3].

Human-to-human transmission is estimated to be prevalent in India and Bangladesh, accounting for 75% and 51% of cases, respectively, in 2004. Currently, there are no vaccines or antiviral drugs available for NiV disease, with the only treatment being supportive care [4]. To prevent further infections, the development of effective vaccines and/or therapeutics is indeed a necessity at this time.

A number of vaccine candidates have demonstrated complete protection against NiV disease in preclinical testing using small animal and nonhuman primate models. Additionally, several NiV vaccine trials have been

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conducted, with the NiV envelope proteins F (fusion) and G (glycoprotein) selected for vaccine development based on our previous understanding of immunity to other paramyxoviruses [5].

There are currently several immunoinformatic techniques available for predicting B and T cell epitopes with excellent sensitivity and specificity. These techniques are essential for understanding the molecular basis of immunity and for developing epitope-based peptide vaccines. In this study, we employed reverse vaccinology analysis to predict B-cell epitopes for vaccine development against the *Nipah virus*.

MATERIAL AND METHODS

Sample Retrieval from Database

The NiV fusion (F) protein (RefSeq. NP_112026.1) was obtained from the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/) [6].

The Screening of Conserved Domain

The 3D structure of the conserved domain in the NiV fusion protein sequences was modeled using SWISS-MODEL (https://swissmodel.expasy.org) with a homology modeling approach [7].

Immunoinformatics Prediction

This study involved the prediction of linear B-cell epitopes using tools such as BepiPred, Emini Surface Accessibility, and Kolaskar-Tongaonkar antigenicity available on the Immune Epitope Database Analysis Resource (IEDB-AR) webserver (http://tools.iedb.org/main/bcell) [8]. We predicted the characteristics of candidate epitopes that can act as protective antigens using the VaxiJen v2.0 web server (http://www.ddgpharmfac.net/vaxijen/VaxiJen/VaxiJen.html) [9].

The toxicity and allergenicity of the peptides were predicted using the ToxinPred online tool (http://crdd.osdd.net/raghava/toxinpred/) and the AllerTOP v2.0 tool (https://www.ddg-pharmfac.net/AllerTOP/), respectively. These tools were also employed to predict the toxicity and allergenicity of the bioactive peptides. [9,10]. Prediction of similarity with proteins in Homo sapiens cells is performed on vaccine candidate peptides using the Basic Local Alignment Protein Search Tool (BLASTp) (https://blast.ncbi.nlm.nih.gov/Blast.cgi).

Protein Docking

The protein docking process was employed to determine the molecular interaction between the ligand and the receptor. In this study, the candidate epitope from the NiV fusion (F) protein was bound to B-cell receptors (BCR). The receptor was obtained from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB) (https://www.rcsb.org/) [12]. Peptides must be converted into PDB format before their structures can be predicted using the PEPFOLD-3 webserver https://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3) [13]. PatchDock is also used to predict the structure of protein–protein and protein–small molecule complexes.(https://bioinfo3d.cs.tau.ac.il/PatchDock/php.php) [14].

Docking is a technique for elucidating fundamental biological processes and characterizing the behavior of small molecules in the binding sites of target proteins. [15]. The results were visualized in PyMol software to provide a three-dimensional (3D) representation of the proteins. [16].

Results and Discussion

Conserved Identification from NiV fusion protein

A fusion protein of Nipah virus sequences was used in this study, and it had previously been obtained from the NCBI web server. The reference sequences are protein sequences from Nipah henipavirus. The sequences, which have a length of 546 amino acids, have been obtained from NCBI and can be seen as follows:

"MVVILDKRCYCNLLILILMISECSVGILHYEKLS
KIGLVKGVTRKYKIKSNPLTKDIVIKMIPNVSNMSQ
CTGSVMENYKTRLNGILTPIKGALEIYKNNTHDLVG
DVRLAGVIMAGVAIGIATAAQITAGVALYEAMKNA
DNINKLKSSIESTNEAVVKLQETAEKTVYVLTALQD

YINTNLVPTIDKISCKQTELSLDLALSKYLSDLLFVF
GPNLQDPVSNSMTIQAISQAFGGNYETLLRTLGYAT
EDFDDLLESDSITGQIIYVDLSSYYIIVRVYFPILTEIQ
QAYIQELLPVSFNNDNSEWISIVPNFILVRNTLISNIEI
GFCLITKRSVICNQDYATPMTNNMRECLTGSTEKCP
RELVVSSHVPRFALSNGVLFANCISVTCQCQTTGRA
ISQSGEQTLLMIDNTTCPTAVLGNVIISLGKYLGSVN
YNSEGIAIGPPVFTDKVDISSQISSMNQSLQQSKDYI
KEAQRLLDTVNPSLISMLSMIILYVLSIASLCIGLITFI
SFIIVEKKRNTYSRLEDRRVRPTSSGDLYYIGT"

The sequences were modeled using the SWISS-MODEL web server. Three-dimensional (3D) modeling is required to identify the structure of the protein to be utilized, which will subsequently assist in the identification process.

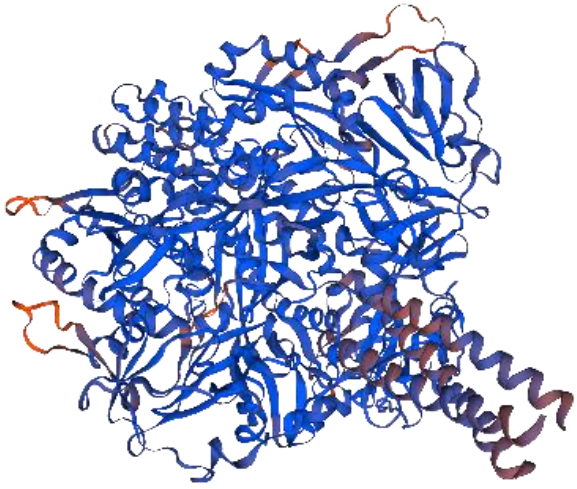

Fig. 1. The conserved domain on fusion proteins was analyzed for the potential of B-cell immunogenicity to obtain specific 3D structure of fusion protein Nipah virus modelled using SWISS-MODEL.

The B-cell Immunogenicity Predictions of Peptide Vaccine Candidate

The conserved domain on fusion proteins was analyzed for the potential of B-cell immunogenicity to obtain specific peptides as vaccine candidates. In our study, we predicted the B-cell epitopes of the conserved region of the fusion protein based on BepiPred, Emini Surface Accessibility, and Kolaskar–Tongaonkar antigenicity using the IEDB.

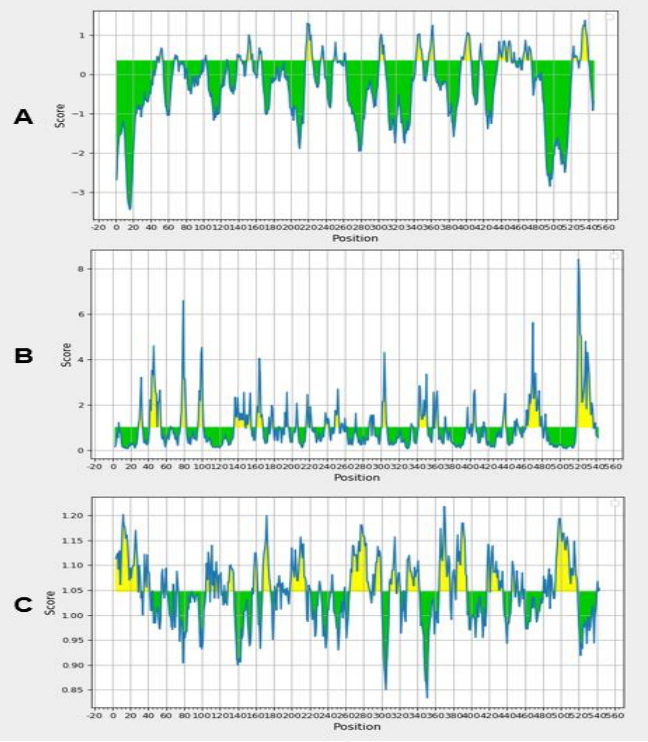

Fig. 2. Prediction of B cell epitopes by different parameters. (A) BepiPred, (B) Emini surface accessibility, (C) Kolaskar–Tongaonkar antigenicity. B cell epitope prediction was done using the IEDB server. The yellow region was a positive prediction of B cell epitope, whereas the green region was negative.

These methods were used to predict specific areas in proteins that bind to the B cell receptor, and these areas must be on the surface and immunogenic (Fig. 2). Bepipred was used to predict linear B-cell epitopes, resulting in 4 epitopes with a minimum propensity of -0.006, a maximum score of 1.381, and a threshold of 0.350. Emini Surface Accessibility was used to predict the surface accessibility of a protein, yielding 8 peptides with a minimum propensity of 0.047, a maximum score of 8.418, and a threshold of 1.000. Kolaskar-Tongaonkar was used to predict antigenic determinants on the protein, resulting in 5 peptides with a minimum propensity of 0.834, a maximum score of 1.218, and a threshold of 1.047. We predicted the antigenicity of the peptides using VaxiJen v2.0 and obtained peptides with antigenic properties. Furthermore, the peptides were analyzed for their similarity to cell surface receptors in Homo sapiens using the BLASTp server.

TABLE I. PREDICTION OF B CELL EPITOPES USING BEPIPRED

No	Peptide	VaxiJen 2.0	AllerTOP	ToxinPred	Similarity
1	QTTGRAISQSGE	Antigen	Non-Allergen	Non-Toxin	< 40
2	QSLQQSKDYIK	Non - Antigen	Non-Allergen	Non-Toxin	No Similaritites
3	NYNSEGIAIG	Antigen	Allergen	Non-Toxin	No Similaritites
4	RRVRPTSSGD	Antigen	Allergen	Non-Toxin	<40

TABLE II. PREDICTION OF B CELL EPITOPES USING EMINI SURFACE ACCESSIBILITY

No	Peptide	VaxiJen 2.0	AllerTOP	ToxinPred	Similarity
1	MIILYVLSIASLCIGLITFISFIIV	Antigen	Non-Allergen	Non-Toxin	80-200
2	SLDLALSKYLSDLLFVFGP	Non - Antigen	Non-Allergen	Non-Toxin	< 40
3	FANCISVTCQCQ	Antigen	Allergen	Non-Toxin	< 40
4	ILDKRCYCNLLILILMISECSVGIL	Antigen	Allergen	Non-Toxin	< 40
5	QIIYVDLSSYYIIVRVYFPILTE	Non - Antigen	Non-Allergen	Non-Toxin	No Similaritites
6	GFCLITKRSVICNQ	Antigen	Allergen	Non-Toxin	No Similaritites
7	LGNVIISLGKYLGS	Non - Antigen	Non-Allergen	Non-Toxin	40 - 50
8	RELVVSSHVPRF	Antigen	Non-Allergen	Non-Toxin	< 40

TABLE III. PREDICTION OF B CELL EPITOPES USING KOLASKAR – TONGAONKAR ANTIGENICITY

No	Peptide	VaxiJen 2.0	AllerTOP	ToxinPred	Similarity
1	EKKRNTYSRLEDRRVRPTS	Antigen	Non-Allergen	Non-Toxin	50 - 80
2	MNSQSLQQSKDYIKEAQRL	Non - Antigen	Non-Allergen	Non-Toxin	No Similaritites
3	VTRKYIKSNPLT	Antigen	Allergen	Non-Toxin	No Similaritites
4	EAMKNADNINKLK	Non-Antigen	Allergen	Non-Toxin	40 - 50
5	QDYATPMTNNM	Non-Antigen	Allergen	Non-Toxin	No Similaritites

The screening identified B-cell epitopes of NiV fusion protein peptides that met the criteria for vaccine candidates, and these epitopes are highlighted in Table IV. These candidates can be further evaluated using a molecular docking method for selection.

TABLE IV. PEPTIDES CANDIDATE

Molecular Complex	Peptide	Receptor	Global Energy (kcal/mol)
Pep_A	QTTGRAISQSGE	5IFH	-48.18
Pep_B	MIILYVLSIASLCIGLITFISFIIV	5IFH	-47.05
Pep_C	SLDLALSKYLSDLLFVFGP	5IFH	-34.17
Pep_D	FANCISVTCQCQ	5IFH	-49.70
Pep_E	EKKRNTYSRLEDRRVRPTS	5IFH	1.24

The peptides showed no similarity to the cell surface receptors of Homo sapiens, with a score of less than 20%, suggesting that they are unlikely to cause autoimmune reactions [17].

Molecular Interaction between Peptide-BCR

Peptides meeting the criteria for B-cell immunogenicity were modeled via PEP-FOLD using fold recognition [18]. Subsequently, the structures were obtained and stored in .pdb format. Next, 3D samples of the antigen-binding fragment BCR receptor (ID 5IFH) were retrieved from the RCSB database. Protein-peptide docking simulations using PatchDock were performed to determine binding energy, which is crucial for forming stable complexes and activating biological responses at BCR receptors[14]. The results display the binding energy generated by all the ligands and are visualized in PyMol software using structural and color selection (Fig. 3).

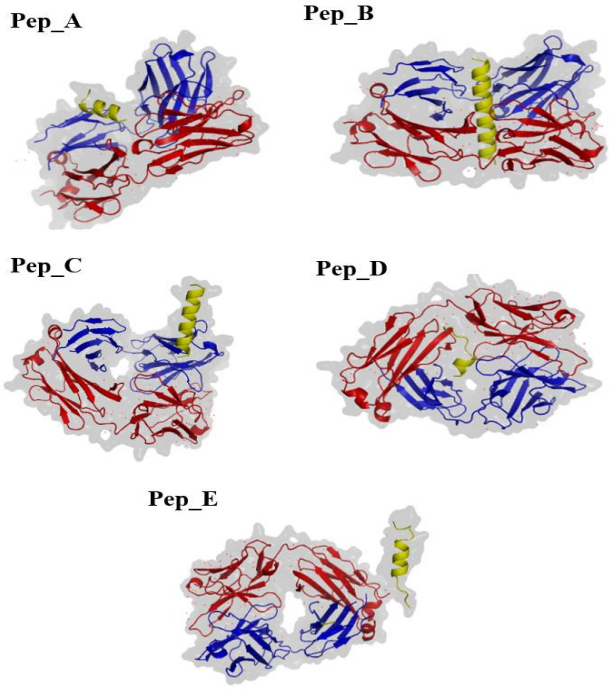

Fig. 3. Visualization of the bond between the ligand and the receptor of peptides candidate

The analysis revealed that Pep_D FANCISVTCQCQ is the most promising vaccine candidate against the Nipah virus. Pep_D holds significant potential as an epitope-based peptide vaccine candidate due to its exceptionally low binding energy score, facilitating the formation of molecular complexes [19,20,21,22].

CONCLUSION

In conclusion, the analysis of B-cell epitopes in the fusion protein NiV peptides has identified five candidates with varying global energy values. We recommend Pep_D FANCISVTCQCQ as a promising epitope-based peptide vaccine candidate against the Nipah virus due to its high immunogenicity and its non-triggering of autoimmune mechanisms. Pep_D has demonstrated the ability to form molecular complexes with the lowest binding energy, facilitating transduction signal activation and direct activation of the B-cell immune response. Nevertheless,

further research into NiV vaccine development should prioritize conducting in vivo and in vitro studies to validate the vaccine's performance.

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تحليل اللقاحات العكسي لخلايا B ضد فيروس نيباه باستخدام بروتين الانصهار

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ملخص

فيروس نيباه (NiV) هو فيروس باراميكسوفيروس ممرض لفيروس الحمض النووي الريبي ويسبب أمراضًا تنفسية غير مميتة في الخنازير التي تم الإبلاغ عنها في الأصل في ماليزيا في عام 1998. ويعتبر فيروس نيبا سببًا محتملاً لتفشي المرض لأنه حيواني المنشأ. ومع ذلك، لا توجد لقاحات أو عقاقير مضادة للفيروسات موجودة ضد النيكل. لذلك، فإن الهدف الرئيسي هو تطوير لقاحات فعالة من خلال توصيف بروتين الاندماج من NiV. استخدمنا التسلسل المرجعي الذي تم استرداده من المركز الوطني لمعلومات التكنولوجيا الحيوية ((NCBI، ثم تم تصميمه بنمذجة ثلاثية الأبعاد للحصول على المنطقة المحفوظة لبروتين الاندماج. تم تقييم التفاعل بين المنطقة المحفوظة مع مستقبلات الخلايا البائية من خلال نهج الالتحام الجزيئي. تم التعرف على حاتمة الخلية B باستخدام خادم الويب لقاعدة البيانات الحلقية المناعية (IEDB). نتيجة لذلك، نوصي بـ Pep_D FANCISVTCQCQ باعتباره لقاح ببتيد قائم على الحاتمة ضد فيروس نيباه. يعتبر Pep D عالي المناعة ولا يسبب تفاعلات المناعة الذاتية. يحتوي Pep D على أقل طاقة ملزمة للمجمعات الجزيئية BCR التي يمكنها تنشيط إشارة التحويل والاستجابة المناعية المباشرة للخلايا B. ومع ذلك، هناك حاجة إلى مزيد من الدراسة للتأكيد (في المختبر وفي الجسم الحي).

الكلمات الدالة: بروتين الاندماج، فيروس نيباه، التطعيم العكسي، المعلومات المناعية.

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Evaluation of Blood Pressure in Children Treated with Ceftriaxone: A Case-Control Study

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ABSTRACT

Background: In children, high blood pressure can develop into hypertension and its consequences during puberty and adulthood. High blood pressure in children is often secondary to other causes, including renal diseases. Nephrolithiasis is one of the causes of secondary hypertension. The extensive use of cephalosporins in hospitals, particularly ceftriaxone, can result in nephrolithiasis. Therefore, the purpose of this study was to assess the relationship between ceftriaxone treatment and elevated blood pressure in children.

Method: The research was conducted as a case-control study over an 18-month period from 2018 to 2019. In this study, blood pressure was measured in 111 children aged 3-13 years who were hospitalized at Amir Kabir Hospital in Arak and received ceftriaxone for at least 48 hours. As a control group, 111 children who did not receive ceftriaxone had their blood pressure measured. The blood pressure levels and percentiles of children in the two groups were then compared.

Result: In the case and control groups, the mean age was 5.1±1.61 and 6.04±2.4 years, and the mean height was 109.17±10.71 and 114.86±12.95 cm, respectively. A slightly higher mean systolic blood pressure percentile was observed in the case group (65.59±18.17) than in the control group (65.28±14.51) (P=0.112), and the mean diastolic blood pressure percentile was also slightly higher in the case group (58.89±18.88) than in the control group (54.85±19.28) (P=0.317). The difference in diastolic blood pressure was greater than in systolic blood pressure. However, these detected differences were slight and not statistically significant.

Conclusion: This study showed no association between blood pressure levels and ceftriaxone treatment in children older than three years who received the medicine for at least 48 hours. However, additional research is suggested, focusing on the effects of the medicine at higher doses and over a longer period of time following administration.

Keywords: ceftriaxone; hypertension; blood pressure; nephrolithiasis; children.

1. INTRODUCTION

The global age-standardised prevalence of hypertension in adults in 2019 was estimated to be 32% in women and 34% in men (1). However, children and adolescents have a lower prevalence of hypertension than adults (2). Several population-based and school-based screening studies indicate that the prevalence of 95th percentile hypertension in children increased from the late 1980s to the early 2000s (3). Evidence suggests that hypertension persists from childhood into adulthood, making it increasingly important to manage elevated blood pressure in children and adolescents (4). In children, systemic hypertension is uncommon, and its

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prevalence is less than 1%, but if present, it often indicates the course of the primary disease (secondary hypertension) (5). The incidence and potential causes of secondary hypertension vary with age. Renal parenchymal disease and coarctation of the aorta are the most common causes in children (6, 7). Blood pressure should be measured routinely in all children over three, either during routine care or during emergency visits (8).

Adult hypertension is defined as blood pressure of 130/80 mmHg or above, regardless of body size, gender, or age (9, 10). This clinical definition is based on the findings of large studies on the influence of antihypertensive drugs on the risk of cardiovascular diseases and mortality and establishes a connection between blood pressure and the risk of cardiovascular events (11). This definition is inapplicable to children since cardiovascular events, except for ventricular hypertrophy, do not usually occur until adulthood (5). Thus, the definition of hypertension in childhood is based on the distribution of normal blood pressure levels in healthy children (12).

Body size is the most influential factor in determining blood pressure levels in children and adolescents. For a more precise classification of blood pressure levels based on the normal growth rate of children, numerous variables, including height, age, and gender, have been considered (5, 13).

Cephalosporins are the most frequently used beta-lactam class and one of the most commonly used antibiotics for treating common infections, and their use has increased over time (14, 15). Ceftriaxone, a third-generation cephalosporin, is a low-risk drug with a longer serum half-life than other cephalosporins, often eliminating the need for repeated injections (16). Due to these features, ceftriaxone is one of the most commonly used medicines in this class. Ceftriaxone has been approved for the treatment of certain types of bacterial meningitis, as well as severe infections caused by penicillin-resistant pneumococcal strains (17).

The common side effects of cephalosporins are divided into two categories: Allergies and toxicity (18). The majority of cephalosporins are metabolized in the liver, and the main route of excretion is renal via active tubular secretion. Renal excretion of ceftriaxone is approximately 33-67%, with the residue being excreted in the bile. Because ceftriaxone appears to have less renal excretion, its renal complications and toxicity are reduced (14, 17). However, renal disorders with ceftriaxone have been documented, including renal stones and nephrolithiasis (19-21), and acute renal failure (22, 23).

Numerous studies have demonstrated an association between nephrolithiasis and hypertension (24). Due to the potential risk of nephrolithiasis, long-term ceftriaxone users are likely to develop hypertension. However, no research has yet studied the relationship between ceftriaxone administration and blood pressure. The purpose of this study was to determine the association between ceftriaxone use and elevated blood pressure in children, as well as to monitor blood pressure in children treated with ceftriaxone.

2. MATERIAL AND METHOD

Study design and patient recruitment

This case-control study included 222 children who were admitted to Amirkabir Hospital in Arak over the course of 18 months between 2018 and 2019. Of these, 111 children aged 3-13 years who were hospitalized and treated with ceftriaxone for any reason for at least 48 hours were included in the study, and their blood pressure was measured. This age range was chosen since blood pressure classification based on percentile is applicable until the age of 13 (25). All these patients were hospitalized due to pyelonephritis. Another group of 111 children aged 3-13 years without specific diseases, who visited the hospital for outpatient care or came to the hospital as patient companions during visiting hours and had not taken ceftriaxone, were also measured for their blood pressure as a control group. The case group members were matched to the control group in terms of gender. Exclusion criteria included the presence or

diagnosis of an underlying or specific condition in the child, as well as the absence of parental consent for participation in the study.

Blood pressure measurement

First, written consent was obtained from the children's parents. All children in both groups rested for at least 30 minutes before their blood pressure was measured. To increase the accuracy of the measurements and reduce possible mistakes, the blood pressure measurements in the case group, who were hospitalized, were repeated twice at intervals of at least 3 hours. Blood pressure was taken from the right arm, as the right arm is more appropriate for hypertension screening (26, 27). Elevated blood pressure was defined as prehypertension (above the 90th percentile and below the 95th percentile), Stage 1 hypertension (above the 95th percentile and below the 99th percentile + 5 mmHg), and Stage 2 hypertension (above the 99th percentile + 5 mmHg) (28) in both groups. If the blood pressure was above the 95th percentile, the blood pressure in the left arm was also measured. In the event of abnormal blood pressure, parents were asked to return to the clinic two weeks later for a second blood pressure measurement. This was done because pyelonephritis can also induce hypertension, and after the completion of the treatment period (14 days), this elevation in blood pressure will resolve as a result of the disease's treatment. This was performed in both the case and control groups. Three sphygmomanometers of varying sizes were used for measurements based on the size of the child's arm. Simultaneously with blood pressure measurements, the children's age, gender, and height were recorded. The measured blood pressure values were classified based on age, gender, and height into the relevant blood pressure percentiles. The height percentile was calculated for all children based on their age and height using the growth charts available on the "Centres for Disease Control and Prevention" website (29). The tables in the "Fourth Report on the Diagnosis, Evaluation, and Treatment of High Blood Pressure in Children and Adolescents" (28) were used to calculate blood pressure percentiles in all children based on age, gender, and height percentile.

Statistical analysis

After entering the data into SPSS software version 23, central and dispersion indices were utilized for statistical analysis. Categorical variables were presented as percentages, and continuous variables were presented as the mean and standard deviation. To evaluate and compare blood pressure values between the case and control groups, data were analysed using the Mann-Whitney U test with a 5% level of significance.

3. RESULTS

The first section of the statistical analysis evaluated the sample's demographic characteristics. In the case group, 58 children (52.3%) were female, and 53 (47.7%) were male. In the control group, 58 (52.3%) were female, and 53 (47.7%) were male. Additional demographic data are presented in Table 1. There were no significant differences between the two groups in terms of demographic parameters ($P > 0.05$).

Table 1. Demographic characteristics

Demographic characteristics	Group	Mean	Standard deviation	Lowest	highest
Age	Case	5.10	1.61	3	10
	Control	6.04	2.04	3	13
Height	Case	109.17	10.71	91	139
	Control	114.86	12.95	92	151
Height percentile	Case	50.22	21	7	98
	Control	48.27	19.88	7	93

In the case group, the mean systolic blood pressure was 98.91 ± 6.64 mmHg (range 80-115), while in the control group, it was 100.14 ± 13.6 mmHg (range 86-117). The mean diastolic blood pressure in the case group was 56.31 ± 7.09 mmHg (range 40-76), and in the control group, it was 56.18 ± 6.69 mmHg (range 43-75). Additionally, the mean systolic blood pressure percentile in the case group was 65.59 ± 18.17 (range 14-94), and in the control group, it was 65.28 ± 14.51 (range 31-95). The mean diastolic blood pressure percentile in the case group was 58.89 ± 18.88 (range 13-94), and in the control group, it was 54.85 ± 19.28 (range 19-94).

In the second measurement for the case group, the mean systolic blood pressure was 99.05 ± 6.35 mmHg (range 82-114), and the mean diastolic blood pressure was 56.07 ± 7.20 mmHg (range 40-75). The mean systolic blood pressure percentile was 66.27 ± 16.71 (range 17-97), and the mean diastolic blood pressure percentile was 58.09 ± 18.87 (range 14-91). Notably, the mean and percentile of blood pressure in the case group did not change significantly between the first and second measurement.

Out of the 111 children whose blood pressure was measured twice in the case group, seven had blood pressure levels above the normal range. Four of these children had blood pressure levels above the 90th percentile in both measurements, two in the first measurement, and one in the second measurement. Six out of these seven children with elevated blood pressure had prehypertension, and only one had blood pressure above the 95th percentile, indicating the first stage of hypertension. The left arm blood pressure of the child with blood pressure higher than the 95th percentile was similarly above the 95th percentile. Parents of these seven children were asked to bring their child for blood pressure re-measurement two weeks later, but ultimately, only five of them had their blood pressure re-measured. Two out of these five children continued to have blood pressure above the 90th percentile, whereas the remaining three had blood pressure below the 90th percentile. In the control group, six out of the 111 children whose blood pressure was measured had higher than normal blood pressure. Five children in this group were in the prehypertension stage, and one was in stage 1 hypertension. Two weeks later, blood pressure measurements were repeated for these children, with only two of them returning for re-measurement, and one of these two still had blood pressure above the 90th percentile.

Tables 2 and 3 display blood pressure and blood pressure percentile comparisons between the case and control groups. As shown in the tables, there were no significant differences in blood pressure levels between children who received ceftriaxone and those who did not.

Table 2. Comparison of blood pressure in the two groups based on Mann-Whitney U test

Variable	Group	Mean	Standard deviation	P-value
Systolic blood pressure	Case	98.91	6.64	0.221
	Control	100.14	6.13	
Diastolic blood pressure	Case	56.31	7.09	0.485
	Control	56.81	6.69	

Table 3. Comparison of blood pressure percentile in the two groups based on Mann-Whitney U test

Variable	Group	Mean	Standard deviation	P-value
Systolic blood pressure	Case	65.59	18.17	0.112
	Control	65.28	14.51	
Diastolic blood pressure	Case	58.89	18.88	0.317
	Control	54.85	19.28	

4. DISCUSSION

In the case and control groups, the mean age was 5.1 ± 1.61 and 6.04 ± 2.4 years, respectively, and the mean height was 109.17 ± 10.71 and 114.86 ± 12.95 cm, respectively. Both groups' blood pressure levels and percentiles were compared. According to statistical analysis, the difference in systolic and diastolic blood pressure values between the two groups was not statistically significant at the 5% significance level. However, since blood pressure in children varies with age, gender, and height, the blood pressure percentiles obtained in the two groups were also compared. After adjusting for children's height and age, which were greater in the control group than in the case group ($P > 0.05$), blood pressure percentiles were found to be higher in the case group, although this difference was not statistically significant. This suggests that there may be underlying causes for the observed elevated blood pressure in children receiving ceftriaxone.

Ceftriaxone use can result in renal complications (19, 30, 31). Nephrolithiasis is a possible side effect of ceftriaxone. At therapeutic levels, ceftriaxone crystallizes with calcium in the urine and adheres to the surface of renal tubular cells (32). Approximately 1.4-7.8% of ceftriaxone-treated individuals develop renal calculi within 7 days of completing the normal course of treatment (32, 33). Typically, ceftriaxone renal stones are small, asymptomatic, and require no special therapy. Following discontinuation of ceftriaxone treatment, the stones usually pass naturally, but in some cases, they can be large and cause nephrolithiasis (22). Nephrolithiasis can lead to hypertension. In both nephrolithiasis and hypertension, alterations in calcium metabolism may play a significant role in the pathophysiology (34). Since the use of ceftriaxone is associated with increased urinary calcium excretion (35) and the development of renal stones, an elevation in blood pressure can be expected in patients receiving ceftriaxone. Moreover, urinary tract obstruction caused by severe nephrolithiasis can result in ceftriaxone-associated postrenal acute kidney injury (AKI) (22, 36). AKI is associated with CKD and hypertension (37, 38). As a result, ceftriaxone may cause an elevation in blood pressure in a variety of ways.

One of the factors that may interfere with the diagnosis of the primary cause of elevated blood pressure in children receiving ceftriaxone is the infectious condition for which ceftriaxone is prescribed. Ceftriaxone is administered every 24 hours to hospitalized children with severe pyelonephritis until they are clinically improved and have been fever-free for 24 hours (39). Severe pyelonephritis is the leading cause of acquired renal scarring in childhood, which, in a small but significant proportion of patients, may progress to hypertension (40).

If the medication is effective, this increase in blood pressure will subside. Therefore, in our study, the blood pressure of children with high blood pressure was re-measured after two weeks at the end of the treatment period. Since some children's blood pressure had returned to normal while others still had elevated blood pressure, the impact of this factor cannot be ruled out in this study.

One limitation of the study was that the dose of injectable medicine for children was not considered a variable, and children treated with any drug dose were included. Additionally, the duration of ceftriaxone administration was not compared, and all participants who received ceftriaxone for more than 48 hours were placed in the same group. Another limitation of this study was that all children treated with ceftriaxone in this center were hospitalized due to pyelonephritis. It is recommended that future research include children treated with ceftriaxone for other reasons. To obtain more accurate results, it is also suggested that the study be conducted with a larger sample size and a longer duration of follow-up.

5. CONCLUSION

Although this study did not find a correlation between hypertension in children and ceftriaxone use, it is advisable to avoid prescribing this medication without a clear

medical indication to prevent unnecessary expenses and potential adverse consequences on the kidneys. Furthermore, for future studies, it is recommended to assess blood pressure at various intervals after ceftriaxone treatment. On the other hand, since adverse effects of the medication may become more apparent at higher doses, it is suggested that future research considers evaluating the drug's dosage in addition to the duration of drug use.

Conflicts of Interest

The authors disclose no conflict of interest.

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Ethical approval

Ethical approval was granted by the Ethics Committee of Arak University of Medical Sciences. (Ethical number: IR.ARAKMU.REC.1396.116)**.**

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تقييم ضغط الدم في الأطفال الذين يتلقون علاجًا بالسيفترياكسون: دراسة حالة وشاهد

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ملخص

الخلفية: في الأطفال، يمكن أن يتطور ارتفاع ضغط الدم إلى ارتفاع ضغط الدم ومضاعفاته خلال فترة البلوغ والبلوغ. يكون ارتفاع ضغط الدم في الأطفال غالبًا ثانويًا لأسباب أخرى بما في ذلك الأمراض الكلوية. حصوة الكلية هي واحدة من أسباب ارتفاع ضغط الدم الثانوي. يمكن أن يؤدي الاستخدام المكثف للسيفالوسبورينات في المستشفيات، ولا سيما السيفترياكسون، إلى حصوة الكلية. لذلك، كانت الغاية من هذه الدراسة هي تقييم العلاقة بين علاج السيفترياكسون وارتفاع ضغط الدم لدى الأطفال.

الطريقة: تمت الدراسة كدراسة حالة وشاهد خلال فترة 18 شهرًا بين عامي 2018 و2019. في هذه الدراسة، تم قياس ضغط الدم في 111 طفلاً تتراوح أعمارهم بين 3 و13 عامًا وتم نقلهم إلى مستشفى أمير كبير في عراق وتلقوا السيفترياكسون لمدة 48 ساعة على الأقل. كمجموعة ضابطة، تم قياس ضغط الدم لدى 111 طفلاً لم يتلقوا السيفترياكسون. ثم تم مقارنة مستويات ضغط الدم والنسب المئوية للأطفال في المجموعتين.

النتيجة: في مجموعة الحالات ومجموعة الضابطة، كانت الأعمار المتوسطة 5.1 ± 1.61 و6.04 ± 2.4 سنة، وكانت الأطوال المتوسطة 109.17 ± 10.71 و114.86 ± 12.95 سم. لوحظت نسبة متوسطة أعلى لضغط الدم الانقباضي في مجموعة الحالات (65.59 ± 18.17) مقارنة بمجموعة الضابطة(P = 0.112) (14.51 ± 65.28) ، وكانت نسبة ضغط الدم الانبساطي المتوسطة أيضًا أعلى في مجموعة الحالات (58.89 ± 18.88) مقارنة بمجموعة الضابطة (P = 0.317). (19.28 ± 54.85)كان الاختلاف في ضغط الدم الانبساطي أكبر من ضغط الدم الانقباضي. ومع ذلك، فإن الاختلافات المكتشفة طفيفة وغير ذات دلالة إحصائية.

الاستنتاج: أظهرت هذه الدراسة عدم وجود ارتباط بين مستويات ضغط الدم وعلاج السيفترياكسون في الأطفال الذين تزيد أعمارهم عن ثلاث سنوات والذين تلقوا الدواء لمدة لا تقل عن 48 ساعة. ومع ذلك، يُقترح إجراء بحوث إضافية تركز على تأثيرات الدواء عند جرعات أعلى وعلى مدى فترة أطول بعد الإعطاء.

الكلمات الدالة: سيفترياكسون؛ ارتفاع ضغط الدم؛ ضغط الدم؛ حصوات الكلى؛ الأطفال.

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Safety of Adalimumab: An Analysis of the FDA Adverse Event Reporting System (FAERS) Database

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ABSTRACT

Objective: This study aims to assess the safety profile of adalimumab and its biosimilars for each approved indication by analyzing adverse events (AEs) reported in the FDA Adverse Event Reporting System (FAERS) database.

Method: We conducted a retrospective pharmacovigilance analysis of AE reports documented from 2002 to 2022 in the FAERS database. This analysis included descriptive statistics and binary logistic regression analyses. We calculated reporting odds ratios (RORs) with 95% confidence intervals (CI) to investigate safety signals related to the disproportionate reporting of serious AEs for adalimumab and its biosimilars compared to currently available biological products for the same proposed indications.

Results: A total of 543,873 AEs related to adalimumab treatment were reported, with 49.8% classified as serious. Hospitalization was the most frequently reported AE. Risk factors associated with serious AEs included age (≥60 years), male sex, and the concurrent use of adalimumab (ROR >1, P<0.05). Adalimumab exhibited a lower risk of serious AEs compared to abatacept, certolizumab, infliximab, or rituximab. Conversely, etanercept and ixekizumab showed lower odds of serious AEs than adalimumab (ROR <1, P<0.05).

Conclusion: In summary, these findings suggest that adalimumab has a well-tolerated safety profile for approved indications when compared to currently available biological alternatives.

Keywords: Adalimumab, Serious adverse events, FAERS database, Rheumatology, Humira.

INTRODUCTION

Adalimumab is a tumor necrosis factor (TNF) blocker indicated for treating several conditions, including rheumatoid arthritis (RA), juvenile idiopathic arthritis (JIA), psoriatic arthritis (PsA), ankylosing spondylitis (AS), Crohn's disease (CD), ulcerative colitis (UC), plaque psoriasis (Ps), hidradenitis suppurativa (HS), and uveitis (UV) [1, 2, 3, 4, 5, 6, 7, 8, 9, 10]. Adalimumab is a biological reference product available under the brand name Humira®. Currently, seven other biosimilar products have been authorized by the U.S Food and Drug Administration (FDA) under the following names: adalimumab-atto (Amjevita®), adalimumab-adbm (Cyltezo®), adalimumab-adaz (Hyrimoz®), adalimumab-bwwd (Hadlima®), adalimumab-afzb (Abrilada®), adalimumab-fkjp (Hulio®), and adalimumab-aqvh (Yusimry®). All biosimilars were found to exhibit similar efficacy, safety, and immunogenicity to adalimumab [11].

It is well-established that anti-TNFs are associated with serious adverse events (AEs) [12]. In addition, the rate of adalimumab-related AEs was found to vary among distinct disease populations [13]. A randomized, double-blind, parallel-group phase III clinical trial assessing adalimumab for treating RA revealed that 6.5% of

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patients experienced serious AEs (mainly infections, 27.7%), resulting in 7.1% of patients discontinuing treatment [14]. However, AEs are uncommon when adalimumab is used to treat pediatric patients with JIA. Indeed, 67% did not experience any AE, and 31% had local injection-site reactions/pain [15]. In patients with PsA, adalimumab was associated with 8.5% of severe AEs, predominantly infection-related AEs (30.7%), which led to treatment discontinuation in 4.6% of patients [16]. A multicenter, randomized, double-blind, placebo-controlled study was undertaken to evaluate the safety and efficacy of adalimumab for treating AS. Only 2.9% of patients had serious AEs. Infection (31.7%), nasopharyngitis (12.5%), injection-site reaction (10.1%), and headache (9.6%) were the most reported AEs [5]. A systemic review of adalimumab tolerability in CD revealed that infection, arthralgia, nasopharyngitis, headache, nausea, fatigue, abdominal pain, pyrexia, injection-site reaction, and influenza occurred in ≥5% of the population [17]. Among patients with UC, the incidence of serious AEs was 4.9%, with infection and gastrointestinal disorders reported most frequently [18]. Overall, 3.1% of patients with serious AEs had received adalimumab to treat Ps. All patients discontinued adalimumab owing to AE severity. Upper respiratory tract infections (34.6%) and hypertension (8.2%) were the most common AEs in patients with Ps [19]. In patients with HS, the risk ratio for adalimumab-related serious AEs was 1.23 (when used weekly) and 1.19 (when used every other week). Patients with HS were found to be 1.09 and 2.84 more likely to develop headaches when used weekly and every other week, respectively. Infection-related AEs were 1.57 more likely to occur when adalimumab was administered every other week. However, the risk of infection-related AEs was low when adalimumab was administered weekly, with a risk ratio <1 [20]. In patients with UV, treatment with adalimumab resulted in the highest incidence of serious AEs (24%), with 18% discontinuing treatment owing to AEs [21]. Accordingly, the US FDA has added a black box warning to the adalimumab leaflet regarding infections and serious malignant AEs.

The FDA Adverse Event Reporting System (FAERS) is a publicly available database that records spontaneous AEs reported to the FDA by pharmaceutical industries, healthcare providers, and consumers [22]. In the present study, we evaluated the safety of adalimumab for all approved indications by analyzing AE reports extracted from the FAERS database as of June 2022.

METHODS

Data source

Study data were derived from the FAERS database for Q4, 2002 through Q2, 2022, which covers the time since adalimumab was first authorized. Adalimumab and its biosimilars were derived from the FDA Purple Book Lists of Licensed Biological Products with Reference Product Exclusivity and Biosimilarity or Interchangeability Evaluations [23].

Procedure

We conducted a retrospective analysis of an adalimumab reference biological product and its biosimilars using AE reports for approved indications during the study period. AE reports for each reference and its biosimilar biological products were identified using both proprietary (Humira®, Amjevita®, Cyltezo®, Hyrimoz®, Hadlima®, Abrilada®, Hulio®, and Yusimry®) and nonproprietary names (adalimumab, adalimumab-atto, adalimumab-adbm, adalimumab-adaz, adalimumab-bwwd, adalimumab-afzb, adalimumab-fkjp, and adalimumab-aqvh). Duplicate reports were excluded from the analysis. Furthermore, AE reports were filtered according to their proposed indications and were excluded from the analysis if they were not used for an approved indication (RA, JIA, PsA, AS, CD, UC, Ps, HS, and UV). AE reports were analyzed for each indication, whether employed as monotherapy or in combination

with other drugs.

In addition, AE reports were subdivided into direct reports (submitted directly to the FDA), expedited reports (for serious and unexpected AEs not included in the product package insert submitted by the manufacturer), and non-expedited reports (periodic reports included in the package insert submitted by the manufacturer). AE reports were further categorized into serious and non-serious AEs. Serious AEs were defined as death, life-threatening events, disability, congenital anomaly, hospitalization, necessitating intervention, or other serious AE.

Intervention

The interventions were biological products with the same indication(s). Information on the available biological alternatives relevant to the same proposed indications was obtained from the FDA-approved label. The present study included all reports in the FAERS public database, starting from the date the drug was approved until Q2 2022. The FAERS reports were searched using proprietary (Orencia®, Cimzia®, Enbrel®, Erelzi®, Eticovo®, Remicade®, Avsola®, Inflectra®, Ixifi®, Renflexis®, Rituxan®, Riabni®, Ruxience®, Truxima®, and Taltz®) and nonproprietary names (abatacept, certolizumab pegol, etanercept, etanercept-szzs, etanercept-ykro, infliximab, infliximab-axxq, infliximab-dyyb, infliximab-qbtx, infliximab-abda, rituximab, rituximab-arrx, rituximab-pvvr, rituximab-abbs, and ixekizumab).

Statistical analysis

Descriptive statistics were used to assess the characteristics of all reports. A multivariate logistic regression model, adjusted for age (<60 *vs*. ≥60 years), sex (male *vs*. female), and the number of therapies used (monotherapy *vs*. combination therapy), was used to define the variable(s) that could be associated with serious AEs. Disproportionality analysis using reporting odds ratios (RORs) with 95% confidence intervals (CI) was performed to assess the possible association between drug exposure and the odds of serious AEs related to the adalimumab reference and its biosimilars when compared with available biological alternatives for each indication. The significance level was set at $P<0.05$. Statistical analyses were performed using Microsoft Excel and the R Project for Statistical Computing version R x64 4.0.5.

RESULTS

In total, 543,872 adalimumab-related AEs were documented on the FAERS platform between December 31, 2002, and June 15, 2022. RA, CD, and Ps had the highest number of reports, with 187,966 (34.6%), 123,274 (22.7%), and 71,891 (13.2%) reports, respectively. Overall, 51,792 (9.5%) reports were associated with PsA, 31,576 (5.8%) with UC, and 25,829 (4.7%) with AS. During this period, there were only 8,913 (1.6%) reports related to HS, 4,667 (0.9%) to JIA, and 3,286 (0.6%) to UV in the FAERS database (Table 1).

Table 1. Characteristics of AEs reports associated with adalimumab and its biosimilars from December 2002 to June 2022.

	Indications								
	RA	**JIA**	**PsA**	**AS**	**CD**	**UC**	**Ps**	**HS**	**UV**
AEs Reports_no. (%)	187,966 (34.6%)	4,667 (0.9%)	51,792 (9.5%)	25,829 (4.7%)	123,274 (22.7%)	31,576 (5.8%)	71,891 (13.2%)	8,913 (1.6%)	3,286 (0.6%)
Gender									
Male	34,919 (18.6%)	1,194 (25.6%)	18,752 (36.2%)	12,068 (46.7%)	44,984 (36.5%)	13,222 (41.9%)	31,455 (43.8%)	2,052 (23.0%)	877 (26.7%)
Female	146,502 (77.9%)	3,107 (66.6%)	31,808 (61.4%)	13,217 (51.2%)	75,497 (61.2%)	17,638 (55.9%)	38,505 (53.6%)	6,087 (68.3%)	2,278 (69.3%)
Not Specified	6,545 (3.5%)	366 (7.8%)	1,232 (2.4%)	544 (2.1%)	2,793 (2.3%)	716 (2.3%)	1,931 (2.7%)	774 (8.7%)	131 (4.0%)
Age (years)									
<60	63,108 (33.6%)	3155 (67.6%)	22,994 (44.4%)	13,976 (54.1%)	68,120 (55.3%)	16,059 (50.9%)	33,383 (46.4%)	3,542 (39.7%)	1,366 (41.6%)
≥60	67,092 (35.7%)	56 (1.2%)	11,008 (21.3%)	3,317 (12.8%)	16,912 (13.7%)	5,668 (18.0%)	15,696 (21.8%)	472 (5.3%)	527 (16.0%)
Not Specified	57,766 (30.7%)	1,456 (31.2%)	17,789 (34.3%)	8,536 (33.0%)	38,242 (31.0%)	9,849 (31.2%)	22,811 (31.7%)	4,899 (55.0%)	1,393 (42.4%)
Combination Therapy	95,249 (50.7%)	2,403 (51.5%)	30,052 (58.0%)	12,316 (47.7%)	54,033 (43.8%)	14,944 (47.3%)	35,889 (49.9%)	2,508 (28.1%)	1,928 (58.7%)
Reporter Type									
Consumer	120,839 (64.3%)	2,723 (58.3%)	39,409 (76.1%)	19,256 (74.6%)	91,688 (74.4%)	25,012 (79.2%)	51,365 (71.4%)	6,377 (71.5%)	2,487 (75.7%)
Healthcare Professional	55,889 (29.7%)	1,817 (38.9%)	10,877 (21.0%)	5,895 (22.8%)	27,493 (22.3%)	6,253 (19.8%)	18,477 (25.7%)	2,504 (28.1%)	777 (23.6%)
Not Specified	11,238 (6.0%)	127 (2.7%)	1,506 (2.9%)	678 (2.6%)	4,093 (3.3%)	311 (1.0%)	2,049 (2.9%)	32 (0.4%)	22 (0.7%)
Case Priority									
Direct	8,053 (4.3%)	201 (4.3%)	2,152 (4.2%)	1,051 (4.1%)	3,977 (3.2%)	1,065 (3.4%)	3,281 (4.6%)	911 (10.2%)	100 (3.0%)
Expedited	90,084 (47.9%)	2,391 (51.2%)	21,461 (41.4%)	14,297 (55.4%)	55,847 (45.3%)	13,694 (43.4%)	26,152 (36.4%)	4,435 (49.8%)	2,318 (70.5%)
Non-Expedited	89,829 (47.8%)	2,075 (44.5%)	28,179 (46.1%)	10,481 (40.6%)	63,450 (51.5%)	16,817 (53.3%)	42,458 (59.1%)	3,567 (40.0%)	868 (26.4%)
Serious AEs	98,489 (52.4%)	2,545 (54.5%)	23,862 (46.1%)	15,088 (58.4%)	61,586 (50.0%)	15,052 (47.7%)	29,559 (41.1%)	4,847 (54.4%)	2,349 (71.5%)
Hospitalized	39,559 (21.0%)	1,198 (25.7%)	9,828 (19.0%)	6,040 (23.4%)	34,181 (27.7%)	7,863 (24.9%)	12,941 (18.0%)	1,796 (20.2%)	721 (21.9%)
Life-Threatening	2,205 (1.2%)	108 (2.3%)	535 (1.0%)	273 (1.1%)	830 (0.7%)	278 (0.9%)	686 (1.0%)	52 (0.6%)	32 (1.0%)
Disabled	4,775 (2.5%)	181 (3.9%)	958 (1.8%)	662 (2.6%)	915 (0.7%)	278 (0.1%)	953 (1.3%)	91 (1.0%)	70 (2.1%)
Congenital Anomaly	109 (0.1%)	3 (0.1%)	28 (0.1%)	16 (0.1%)	89 (0.1%)	30 (0.1%)	43 (0.1%)	12 (0.1%)	2 (0.1%)
Required Intervention	592 (0.3%)	2 (0.0%)	37 (0.1%)	11 (0.0%)	46 (0.0%)	5 (0.0%)	32 (0.0%)	3 (0.0%)	3 (0.1%)
Death	8,614 (4.6%)	55 (1.2%)	1,241 (2.4%)	788 (3.1%)	2,701 (2.2%)	824 (2.6%)	2,323 (3.2%)	201 (2.3%)	83 (2.5%)
Other Outcomes	67,501 (35.9%)	1,664 (35.7%)	17,053 (32.9%)	10,527 (40.8%)	37,754 (30.6%)	9,366 (29.7%)	19,749 (27.5%)	3,535 (39.7%)	1,891 (57.5%)
Non-Serious	89,478 (47.6%)	2122 (45.5%)	27,930 (53.9%)	10,741 (41.6%)	61,688 (50.0%)	16,524 (52.3%)	42,332 (58.9%)	4,066 (45.6%)	937 (28.5%)

AEs, adverse events; RA, rheumatoid arthritis; JIA, juvenile idiopathic arthritis; PsA, psoriatic arthritis; AS, ankylosing spondylitis; CD, Crohn's disease; UC, ulcerative colitis; Ps, plaque psoriasis; HS, hidradenitis suppurativa; UV, uveitis.

The majority of reports were associated with female patients for all approved indications. Reports associated with RA were almost equal between patients aged < 60 and ≥ 60 years. In addition, adalimumab and its biosimilars were used more frequently in patients aged < 60 years. However, age was not specified in at least 30% of reports. The drug was used in combination with other drugs in approximately 50% of RA, JIA, PsA, Ps, and UV cases. The combination treatment percentages for AS, UC, and CD were 47.7, 47.3, and 43.8%, respectively. The drug was used mainly as monotherapy to treat HS (28.1% of combination). Considering all indications, consumer reports exceeded those by healthcare professionals. For RA, expedited reports were comparable with non-expedited reports (47.8%). The number of expedited reports was higher than that of non-expedited reports for JIA, AS, HS, and UV. Considering patients with PsA, CD, UC, and Ps, expedited reports were fewer than non-expedited reports. Serious AEs were observed in >50% of patients with RA, JIA, AS, CD, HS, and UV; the remaining patients still experienced a high percentage of serious AEs, with approximately 47.7, 46.1, and 41.1% for UC, PsA, and Ps, respectively. For all the approved indications, hospitalization was the most common AE.

The trend of AE reports over time showed a similar pattern for all indications (Figure 1), with a slight initial increase between 2003 and 2010. Subsequently, there was a significant increase in the number of reports, which peaked in 2016. From 2017 onward, the number of reports decreased drastically, except for RA. In addition, the graph illustrates whether adalimumab and its biosimilars were used as monotherapy or combination therapy.

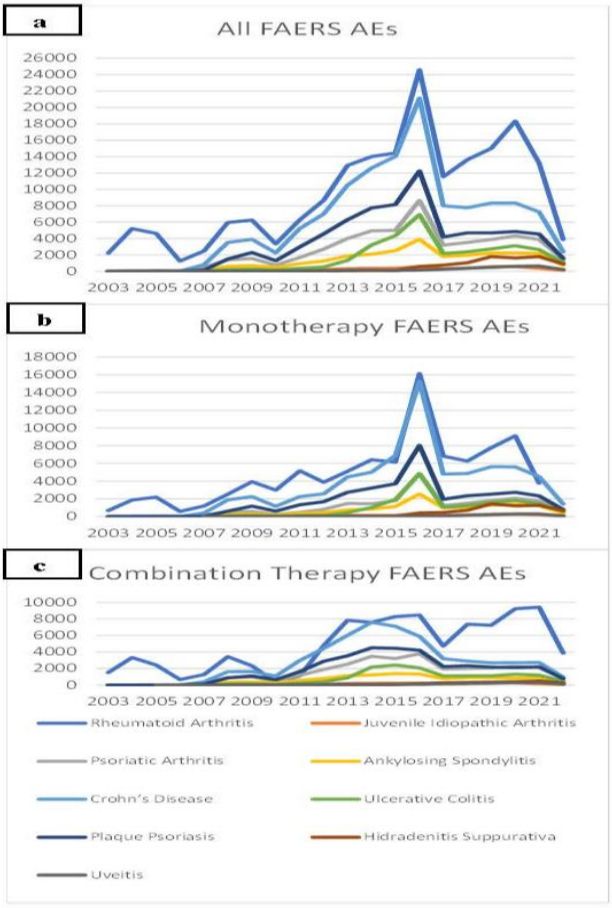

Fig. 1 The number of adalimumab and its biosimilars AE reports for each indication from 2002 to 2022 whenever it was reported (a), reported as monotherapy (b), or in combination (c).

The results of the binary logistic regression showed that patients aged ≥60 years were 1.73 times more likely to experience serious AEs (P < 0.001) (Table 2). In addition, males were 1.30 times more likely to experience serious AEs (P < 0.001). Compared with monotherapy with adalimumab and its biosimilars, patients who received combination therapy were 1.34 more likely to experience serious AEs (P < 0.001).

Table 2. Binary logistic regression for odds of serious outcomes of adalimumab and its biosimilars.

	Serious		
	ROR	**95% CI**	**p-value**
Age			
<60	Ref		
≥60	1.73	1.705–1.756	<0.0001
Gender			
Male	1.30	1.282–1.319	<0.0001
Female	Ref		
Number of therapies			
Monotherapy	Ref		
Combination therapy	1.34	1.318–1.355	<0.0001

ROR: reporting odds ratio; CI: confidence interval; Ref: reference.

Abatacept, certolizumab, etanercept, infliximab, ixekizumab, and rituximab are biological products available for the same proposed indications as adalimumab. Compared with available biological alternatives, the ROR for adalimumab and its biosimilars showed different results depending on the indication and whether the drug was used alone or in combination (Table 3). The ROR for abatacept revealed that adalimumab (whether it was used as monotherapy, combination, or both) was associated with less serious AEs, except when abatacept was used as monotherapy for PsA. Adalimumab was persistently associated with fewer serious AEs than certolizumab, except when employed to treat CD. Etanercept was associated with few serious AEs when used as a monotherapy or in all reports, but the ROR was <1 when used in combination therapy. Infliximab and rituximab RORs were persistently and significantly higher than those of adalimumab for the same indications. Comparing adalimumab with ixekizumab, we noted a ROR >1 in all scenarios, except when adalimumab was used to treat PsA in combination therapy.

Table 3. Disproportionality analysis of adalimumab and its biosimilars compared with the available biological products for the same approved indications.

	All		Monotherapy		Combination Therapy	
Product Name	**ROR (95% CI)**	**p-value**	**ROR (95% CI)**	**p-value**	**ROR (95% CI)**	**p-value**
Adalimumab	Ref		Ref		Ref	
RA						
Abatacept	0.43 (0.419–0.436)	<0.0001	0.61 (0.596–0.624)	<0.0001	0.38 (0.358–0.392)	<0.0001
Certolizumab	0.26 (0.255–0.270)	<0.0001	0.30 (0.293–0.313)	<0.0001	0.25 (0.231–0.266)	<0.0001
Etanercept	1.56 (1.544–1.580)	<0.0001	1.60 (1.576–1.618)	<0.0001	0.84 (0.810–0.863)	<0.0001
Infliximab	0.09 (0.092–0.098)	<0.0001	0.08 (0.077–0.084)	<0.0001	0.24 (0.229–0.257)	<0.0001
Rituximab	0.07 (0.063–0.069)	<0.0001	0.113 (0.107–0.120)	<0.0001	0.10 (0.089–0.105)	<0.0001
JIA						
Abatacept	0.35 (0.298–0.409)	<0.0001	0.80 (0.646–0.981)	0.03	0.29 (0.191–0.438)	<0.0001
Etanercept	2.02 (1.884–2.169)	<0.0001	1.97 (1.816–2.140)	<0.0001	0.84 (0.675–1.055)	**0.14**
PsA						
Abatacept	0.48 (0.428–0.526)	<0.0001	1.41 (1.216–1.642)	<0.0001	0.18 (0.142–0.234)	<0.0001
Certolizumab	0.38 (0.361–0.406)	<0.0001	0.41 (0.386–0.438)	<0.0001	0.37 (0.319–0.438)	<0.0001
Etanercept	1.97 (1.921–2.017)	<0.0001	2.10 (2.041–2.152)	<0.0001	0.84 (0.774–0.901)	<0.0001
Infliximab	0.12 (0.116–0.133)	<0.0001	0.13 (0.119–0.139)	<0.0001	0.20 (0.175–0.231)	<0.0001
Ixekizumab	2.78 (2.548–3.024)	<0.0001	3.84 (3.455–4.626)	<0.0001	0.73 (0.582–0.921)	0.007
AS						
Certolizumab	0.49 (0.454–0.531)	<0.0001	0.50 (0.459–0.540)	<0.0001	0.45 (0.323–0.623)	<0.0001
Etanercept	2.50 (2.409–2.589)	<0.0001	2.74 (2.634–2.842)	<0.0001	0.69 (0.592–0.813)	<0.0001
Infliximab	0.08 (0.069–0.084)	<0.0001	0.07 (0.066–0.082)	<0.0001	0.16 (0.125–0.214)	<0.0001
Ixekizumab	4.68 (3.432–6.383)	<0.0001	5.88 (4.101–8.435)	<0.0001	1.77 (0.723–4.320)	**0.197**
CD						
Certolizumab	1.11 (1.066–1.145)	<0.0001	1.10 (1.062–1.143)	<0.0001	1.13 (0.966–1.330)	**0.131**
Infliximab	0.08 (0.076–0.081)	<0.0001	0.08 (0.074–0.079)	<0.0001	0.19 (0.170–0.215)	<0.0001
UC						
Infliximab	0.08 (0.076–0.085)	<0.0001	0.08 (0.075–0.084)	<0.0001	0.15 (0.128–0.180)	<0.0001
Ps						
Certolizumab	0.24 (0.224–0.265)	<0.0001	0.35 (0.317–0.384)	<0.0001	0.17 (0.128–0.214)	<0.0001
Etanercept	2.14 (2.085–2.187)	<0.0001	2.33 (2.267–2.386)	<0.0001	0.79 (0.721–0.869)	<0.0001
Infliximab	0.06 (0.053–0.064)	<0.0001	0.07 (0.062–0.076)	<0.0001	0.10 (0.085–0.128)	<0.0001
Ixekizumab	2.65 (2.516–2.791)	<0.0001	2.89 (2.730–3.057)	<0.0001	0.93 (0.766–1.135)	**0.518**

ROR, reporting odds ratios; CI, confidence interval; Ref, reference; RA, rheumatoid arthritis; JIA, juvenile idiopathic arthritis; PsA, psoriatic arthritis; AS, ankylosing spondylitis; CD, Crohn's disease; UC, ulcerative colitis; Ps, plaque psoriasis.

Discussion

The anti-TNF agent, adalimumab, was generally well-tolerated. In most cases, adalimumab-related AEs do not necessitate treatment discontinuation. However, serious AEs have also been reported. The most common AEs include infections, injection-site reactions, and malignancy [24, 25]. The present study highlights the safety profile of adalimumab and its biosimilars in patients with RA, JIA, PsA, AS, CD, UC, Ps, HS, and UV. Adalimumab-related AEs were more frequently reported in females (65.7%) than in males (31.3%), consistent with previous findings that females show a higher incidence of several systemic rheumatologic autoimmune diseases than males [26]. Except for RA, adalimumab was mainly used in the < 60-year-old population (44.3%); this could be attributed to five of its indications being approved for the pediatric population (JIA, CD, UC, HS, and UV). Adalimumab-related AEs were mainly reported by consumers (70.5%) rather than healthcare professionals (25.5%), which indicates how incorporating patient assistance can improve the patient safety profile and ensure that measured AEs reflect what matters most to patients. Of the AEs, 50.6% were listed on the package insert (non-expedited). Conversely, 45.3% were serious and unexpected AEs unlisted on the product package insert, primarily for UV (in 70.5% of the AE reports), which could be attributed to the fact that UV was the last indication approved by the FDA in 2016, and the safety profile of adalimumab for this indication is under investigation. Approximately 50% of AE reports were serious (49.8%), and 22.4% required hospitalization.

The number of AE reports was the lowest from 2002 to 2006, given that adalimumab was only approved to treat RA, PsA, and AS during this period. In February 2007, adalimumab was approved for CD. From 2008 to 2016, adalimumab was approved to treat Ps, JIA, UC, HS, and UV [23], which may explain the increased number of reports from 2010 to 2016. Since 2017, the number of AE reports has decreased gradually, as several biological alternatives (reference and biosimilars) have been approved for the same indications as adalimumab, thereby impacting the market share of adalimumab and its biosimilars.

The safety analysis of adalimumab and its biosimilars revealed that age (≥60 years), male sex, and its use in combination are risk factors for serious AEs, corroborating the finding of a previous report [13].

In the present analysis, we found that the ROR of serious adalimumab-related AEs varied depending on the alternative used and the disease treated. This finding is consistent with a study conducted by Cross et al., who found that the same drug could exhibit different safety profiles for different diseases [27]. Considering the treatment of RA, JIA, and PsA, the frequency of serious AEs was significantly lower for adalimumab than that for abatacept, except when adalimumab was used as monotherapy for PsA. Based on certolizumab ROR analysis, adalimumab has a high risk of serious AEs when employed for CD, with less serious AEs observed when used to treat RA, PsA, AS, and Ps. More serious AEs were found to occur when etanercept was used as monotherapy; however, the odds of serious AEs were low when used in combination therapy. Adalimumab was significantly associated with fewer serious AEs than infliximab and rituximab. Ixekizumab was associated with a low number of serious AEs (ROR > 1, where adalimumab is the reference). One exception is ixekizumab, which was used in combination therapy for PsA. A previous study has examined the safety signals of disproportionate reporting of serious AEs associated with adalimumab when compared with currently available biological products. The authors found that adalimumab was associated with a lower incidence of serious AEs than infliximab and certolizumab but with a higher incidence than etanercept [28].

In the present study, we examined safety signals of disproportionate reporting of serious AEs for adalimumab and its biosimilars when compared with those of currently

available biological products for the same proposed indications using a pharmacovigilance analysis approach to evaluate the post-marketing safety of adalimumab.

Limitations

In this analysis, most of the AE reported to the FAERS came from consumers who were not necessarily familiar with medication safety across the continuum of care. Therefore, the outcomes may not be accurate.

We could not identify comorbidities (diseases other than those of interest). These comorbidities may improve the safety profile of adalimumab.

The ROR is a quantitative signal detection method that indicates a potential link to safety problems through a statistical correlation between the drug and AE. However, using ROR can be biased and misleading [29].

Finally, spontaneous AE reports may be biased and do not represent every case reported. Inaccurate estimations and missing information are common, which can affect the outcome [30, 31, 32, 33].

CONCLUSIONS

A significant difference in the signals of disproportionate reporting of serious AE between adalimumab and its biosimilars with the currently available alternatives for the same proposed indications was detected after analyzing the spontaneous AE reports from the FAERS database. However, given the limitations of this study, further research using a head-to-head study design to test the serious AE signals observed in this study is required.

Conflict of Interest

The author declares no conflict of interest.

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سلامة دواء أداليموماب: تحليل قاعدة بيانات نظام الإبلاغ عن الآثار الجانبية

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ملخص

الهدف: تحديد مدى سلامة دواء أداليموماب و بدائله الحيوية لكل إستخدام معتمد من خلال تحليل الأثار الجانبية التي تم الإبلاغ عنها إلى قاعدة بيانات نظام الإبلاغ عن الآثار الجانبية التابعة لإدارة الغذاء و الدواء الأمريكية

الطريقة: قمنا بإجراء تحليل إحصائي رجعي لتقارير الآثار الجانبية الموثقة من عام 2002 الى عام 2022. كما قمنا بمقارنة تقارير الآثار الجانبية لدواء الأدابيموماب و بدائله الحيوية مع البدائل الأخرى المتاحة حاليا لكل إستخدام.

النتائج: بالمجمل، تم الإبلاغ عن 543.873 تقرير لدواء الأداليموماب. من بين هؤلاء كان هناك 49.8٪ من الحالات شديدة الخطورة. الحاجة للذهاب إلى المستشفى كان أكثر الأثار الجانبية شيوعا. الذكور، وكبار السن (<60)، وإستخدام أكثر من دواء في نفس الوقت كانوا أكثر العوامل إرتباطا بالآثار الجانبية شديدة الخطورة .

الخلاصة: تشير نتائج هذه الدراسة إلى أن دواء أداليموماب يعتبر أكثر أمانا مقارنة بالبدائل البيولوجية المتاحة حاليا.

الكلمات الدالة: أداليموماب، الآثار الجانبية شديدة الخطورة، قاعدة ببانات، أمراض الروماتيزم، هوميرا.

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Therapeutic Potential of Traditional Medicinal Plants from Algeria for Treatment of Liver Diseases

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ABSTRACT

The objective of our study is to conduct an ethnobotanical investigation of traditional medicinal plants used by people in El-Oued state, southeast Algeria, for the treatment of liver diseases. We collected data through personal interviews and questionnaires. In total, we conducted interviews with 156 respondents, the majority of whom were aged 50 or older. Our study of medicinal plants used for treating liver diseases led to the discovery of 78 species from 41 families and various genera, including 52% of medicinal plants found in the wild. Approximately 77% of these plants were primarily used in dried form for remedy preparation. The most commonly employed preparation method was decoction. Interestingly, 70% of the participants mentioned Zizyphus lotus (L.) Lam., Silybum marianum L., and Atriplex halimus L. as sources for treating liver illnesses. This investigation revealed that many people in the research region still rely on herbal remedies to treat liver disorders. Moreover, the present study provides valuable ethnobotanical data on medicinal plants, serving as a foundational resource for future extensive research in this field.

Keywords: Ethnobotanical, Pharmacopeia, Medicinal plants, Liver diseases, El-Oued.

INTRODUCTION

The use of medicinal plants has long been recognized for its health benefits and curative properties, and it continues to play a significant role in healthcare, particularly at the therapeutic level[1]. Furthermore, the therapeutic advantages of many medicinal plants, which have been empirically used for millennia, have only been scientifically validated in recent decades. Despite the advancements in synthetic chemistry, medicinal plants have maintained their prominence due to their effectiveness in various therapeutic procedures[2]. The World Health Organization estimates that over 80% of African populations rely on traditional pharmacopoeia for treating health issues. The African continent is rich in medicinal plants, with an abundance of diverse species. Out of the 300,000 plant species documented worldwide, over 200,000 are found in the tropical countries of Africa and possess medicinal propertie[3].

Medicinal plants continue to serve as a source of medical care, particularly in developing countries where modern therapeutic systems may be lacking. These medicinal species constitute a vast and diverse group, containing bioactive substances that are employed not only for immediate liver protection[4,5] but also in the pharmaceutical and cosmetic industries[6].

Algeria, due to its biogeographic position, boasts significant ecological diversity. It is a Mediterranean country with a rich medical tradition rooted in traditional knowledge of medicinal plants. While various studies have

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been conducted, a majority of them have centered around wetlands and their avifauna, with only a few dedicated to the study of flora. Consequently, the available documentation often provides general descriptions of the region, supplemented primarily by inventories of flora and fauna[7].

The knowledge of Algerian flora, particularly the Saharan flora, remains primarily empirical. In this context, an ethnobotanical study seeks to emphasize the role of herbal medicine within the traditional healthcare system for the treatment of liver diseases in El-Oued state, situated in southeastern Algeria.

MATERIALS AND METHODS

Study area

The El-Oued region is a large state located in southeastern Algeria, situated between latitudes 33° to 34° N and longitudes 6° to 8° E. It is approximately 620 km away from the capital, Algiers. The region is bounded by Ouargla, Djelfa, Biskra, Khenchla, Tebessa, and Libya, and to the west, it is delimited by the chott of Oued Righ. To the north, it is bordered by the chotts Merouane, Melghir, and Rharsa, and to the east, it is adjacent to the Tunisian chott El-Djerid (see Figure: 01)[8,9].

The soil in the El-Oued region shares many characteristics with other Saharan soils. It is sandy in texture and structure, has low organic content, and offers high water permeability. Additionally, El-Oued experiences high temperatures and significant temperature fluctuations due to its continental location and proximity to the equator, resulting in scorching summers[8].

Regarding the plant cover in the open study area, there is a notable variation in density and diversity among indigenous plants. These plants are characterized by their rapid growth, small size, and adaptation to the region's specific soil and climatic conditions[9].

Methods:

Face-to-face interviews were conducted exclusively with individuals who possessed knowledge of medicinal plants. The interviews involved over 150 respondents, both male and female, ranging in age from 25 to 60. These respondents had diverse educational backgrounds. The purpose of these interviews was to gather ethnobotanical data on the use of medicinal plants for the treatment of liver diseases, encompassing numerous types of data such: as the local name of plant species, growth forms (wild or cultivated species), parts of plants utilized in the traditional treatment, preparation condition (dry or fresh), preparation procedure of medicinal plants[10,11,12]. To ensure the accuracy of the results, we presented plant specimens to multiple individuals after collecting them. Subsequently, the reported medicinal plant specimens were identified and verified by the expert, Professor Atef Chouikh from the Faculty of Natural and Life Sciences at the University of El Oued, Algeria. The scientific and authorship names of the medicinal plants were cross-referenced and validated using the database available at (www.theplantlist.org).

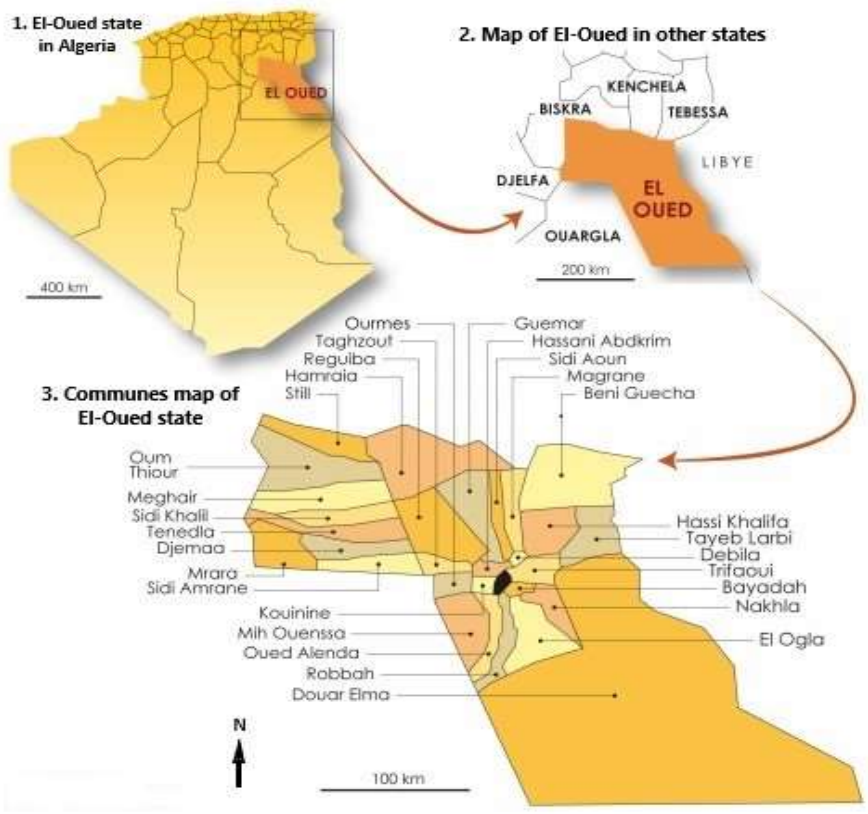

Figure 01: Map of the study area showing the El-Oued region

RESULTS

Demographic details of the informants:

The knowledge results categorized by age, gender, and educational level are presented in Table 01. Among the total informants, the majority (75%) were male, while the remaining (25%) were female due to cultural reasons. Regarding age distribution, the study included participants in the age group ranging from 25 to 60. On average, male informants reported a higher number of medicinal plants compared to female informants. In terms of educational status, the informants varied from (10%) being illiterate to (90%) being literate.

Table 1: Demographic details of the informants.

Parameters	Gender		Age group (In years)		Educational status	
	Male	Female	25-40 Youngers	40-60 Elders	Illiterate	literate
Percentage (%)	75	25	29	71	10	90

Medicinal plants used in the study are:

The data collected identified seventy-eight (78) medicinal plants presented in Table: 02, from forty-one (41) botanical families (Figure: 02), with the Asteraceae, Apiaceae, Lamiaceae, and Brassicaceae families having the highest representation.

Table 2: Medicinal plants used in the study area for treatments.

N°	Scientific name	Family	Local name	Part used
1	*Aegle marmelos* L.	Rutaceae	Kitha hendi	S
2	*Agave americana* L.	Asparagaceae	Hendi	Fr
3	*Ajuga iva* L.	Lamiaceae	Aljeada	St+F
4	*Alchemilla vulgaris* L.	Rosaceae	Rejel alasad	St
5	*Atriplex halimus* L.	Chenopodiaceae	Getaf	L
6	*Ammodavcus leucotrichus* Coss. & Durieu	Apiaceae	Oum deriga	St+S
7	*Anacyclus pyrethrum* L.	Asteraceae	Oude elatas	St
8	*Anagelica officinalis* L.	Apiaceae	Hashishet almalak	F
9	*Aquilaria malaccensis* Lam.	Thymelaeaceae	Shajret ellaud	F
10	*Arctium lappa* L.	Asteraceae	Alarkatioun	R
11	*Artemisia absinthium* L.	Asteraceae	Shajart mariam	F
12	*Artemisia herba alba* Asso.	Asteraceae	Shih	L
13	*Asparagus officinalis* L.	Asparagaceae	Helioun	St
14	*Berberis vulgaris* L.	Berberidaceae	Jarjir elbar	L
15	*Beta vulgaris* L.	Chenopodiaceae	Betraf	R
16	*Brassica oleracea* var *botrytis* L.	Brassicaceae	Shoufleur	Fr
17	*Brassica napus* L.	Brassicaceae	Elefet	R
18	*Brassica oleracea* var *capitata* L.	Brassicaceae	Kromb	Fr
19	*Capparis spinosa* L.	Capparaceae	Shaouklhmar	Ep
20	*Carthamus lanatus* L.	Asteraceae	Zafran	F
21	*Centaurea cacitrapa* L.	Asteraceae	Shaoukejmal	S+Ep
22	*Centaurium erythraea* Rafn.	Gentianaceae	Kantarioun	F+L
23	*Cerasus mahaleb* L.	Rosaceae	Kamha	F
24	*Chamaemelum nobile* L.	Asteraceae	Babounj	F
25	*Chelidonium majus* L.	Papaveraceae	Ergasfar	R
26	*Cicer arietinum* L.	Fabaceae	Homos	L
27	*Cichorium intybus* L.	Asteraceae	Hindeba bary	F+St+L
28	*Citrus limonuna* Osbeck.	Rutaceae	Kars	Fr
29	*Commiphora myrrha* Nees.	Burseraceae	Elmor	St
30	*Camellia sinensis* L.	Theaceae	Tay	L
31	*Convolvulus arvensis* L.	Convolvulaceae	Laway	L
32	*Coriandrum sativum* L.	Apiaceae	Kozbor	L
33	*Cucurbita moschata* Duch.	Cucurbitaceae	Kabouya	Fr
34	*Curcuma longa* L.	Zingiberaceae	Kourkoum	R
35	*Cynara cardunculus* L.	Asteraceae	Khorshof bary	St+F
36	*Cynara scolymus* L.	Asteraceae	Garnoun	Fr
37	*Cyperus esculentus* L.	Cyperaceae	Habelaziz	Ep+R
38	*Daucus carota* L.	Apiaceae	Zrodya	R+S
39	*Ecaballum elaterium* L.	Cucurbitaceae	Fagous lhmir	R+F

N°	Scientific name	Family	Local name	Part used
40	*Equisetum arvense* L.	Equisetaceae	Danab elkhyl	St+L
41	*Ephedra alata* Decne.	Ephedraceae	Alanda	St+L
42	*Eruca sativa* Mill.	Brassicaceae	Jarjir	L
43	*Ficus carica* L.	Moraceae	Karmous	Fr
44	*Foeniculum vuglare* Mill.	Apiaceae	Besbes	R
45	*Fragaria vesca* L.	Rosaceae	Faraoula	Fr
46	*Glycyrrhiza glabra* L.	Fabaceae	Ergesous	L
47	*Helichrysum italicum* Roth.	Asteraceae	Elkhalda	S
48	*Hordeum vulgare* L.	Poaceae	Shair	S
49	*Jasminum grandiflorum* L.	Oleaceae	Yansoun	F
50	*Juniperus oxycedrus* L.	Cupressaceae	Elarar	L
51	*Laurus nobilis* L.	Lauraceae	Raned	L
52	*Linum usitatissimum* L.	Linaceae	Zreat elktan	S
53	*Bunium mauritanicum* L.	Apiaceae	Talghouda	S
54	*Melissa officinalis* L.	Lamiaceae	Mlailisa	L
55	*Mentha spicata* L.	Lamiaceae	Naanaa	L
56	*Spinach officianalis* L.	Amaranthaceae	Salk	L
57	*Nigella sativa* L.	Ranunculaceae	Kamoun asouad	S
58	*Onoprdon matracanthum* L.	Asteraceae	Khourshouf	L
59	*Petroselinum crispum* Mill.	Apiaceae	Maadnous	L
60	*Peumus boldus* Molina.	Monimiaceae	Shajrt elkabed	L+F
61	*Phonix dactylifera* L.	Arecaceae	Tamer	Fr
62	*Phyllanthus niruri* L.	Phyllanthaceae	Elamlaj	Ep
63	*Pistacia lentiscus* L.	Anacardiaceae	Darou	L
67	*Plantago lancelata* L.	Plantaginaceae	Lsan elhamel	Ep
65	*Portulaca oleracea* L	Portulacaceae	Bourtlag	L
66	*Raphanus sativus* L.	Brassicaceae	Fijel	L
67	*Zizyphus lotus* L.	Rhamnaceae	Sider	L
68	*Salvia rosmarinus* Spenn.	Lamiaceae	Eklil eljabal	F+St+L
69	*Salvia officinalis* L.	Lamiaceae	Mayramia	L
70	*Salix fragilis* L.	Salicaceae	Safsaf	St
71	*Senecio vulgaris* L.	Asteraceae	Jedla	St
72	*Silybum marianum* L.	Asteraceae	Shouiket mariam	S
73	*Tamarix articulate* L.	Tamaricaceae	Tarfaya	L
74	*Thymus vulgaris* L.	Lamiaceae	Zaatar	L
75	*Trigonella fornum graecum* L.	Fabaceae	Helba	S
76	*Urtica pillulifera* L.	Urticaceae	Kouraice	S
77	*Verbena officinalis* L.	Verbenaceae	Rejel Elhamam	Ep
78	*Zingiber officinale* Roscoe.	Zingiberaceae	Zanjabil	R

***L**: Leaves, **S**: Seed, **R**: Roots, **F**: Flower, **St**: Stems, **Fr**: Fruits, **Ep**: Entire plant.*

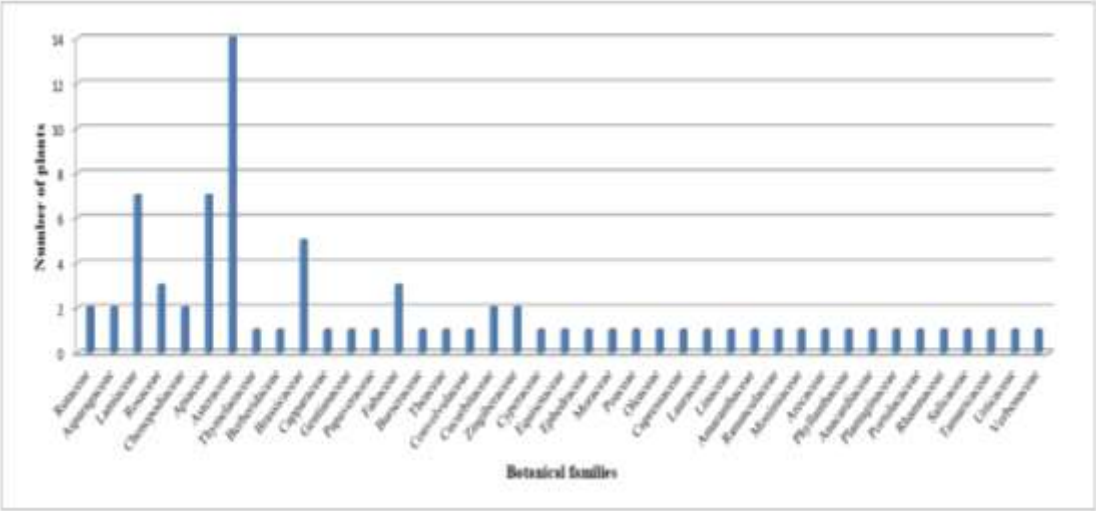

Figure 02: Distribution of medicinal plants used per botanical families in the study area to treat liver diseases.

Growth forms of medicinal plants:

Results obtained for analysis of growth forms of plant species used in El-Oued state for treatment of liver diseases revealed that 52% of plants are wild, and 48% of plants were cultivated species (figure: 03).

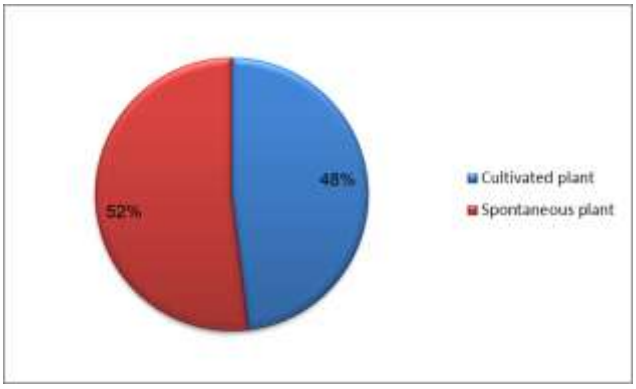

Figure 03: Percentage of growth forms of medicinal plants used in the study area to treat liver diseases.

***Part used and preparation condition of medicinal plants* :**

In the traditional treatment of various liver diseases in El-Oued state, all parts of plants are employed. The vegetable parts used are categorized in descending order of importance as follows: leaves (31%), seeds (15%), roots (14%), flowers (13%), stems (10%), fruits (10%), and the entire plant (7%) (Figure: 04 A). Regarding the preparation condition of medicinal plants, a significant majority (77%) were prepared from dried plant materials, while only a minority (23%) were made from fresh plants (Figure: 04 B).

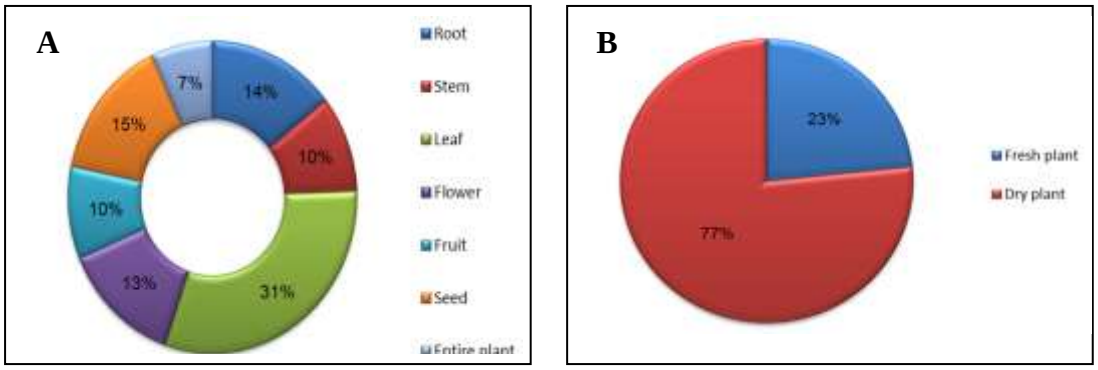

Figure 04: Percentage of parts used (A) and preparation condition of medicinal plants (B) used in the study area to treat liver diseases.

Preparation modes of medicinal plants:

Figure 05 displays the results obtained for the percentage of preparation modes (A) and the percentage of plant extraction methods used for medicinal plants (B) in the El-Oued state for the treatment of liver diseases. In this study, local people in the area use various modes of preparing medicinal plants, such as Extraction (57.14%), Powder (36.45%), Oils (3.44%), Essential Oils (1.97%), and Capsules (0.98%) (Figure: 04 A). Regarding the percentage of plant extraction methods, people primarily use the decoction method (48%), followed by infusion (34%) and maceration (20%) (Figure: 04 B).

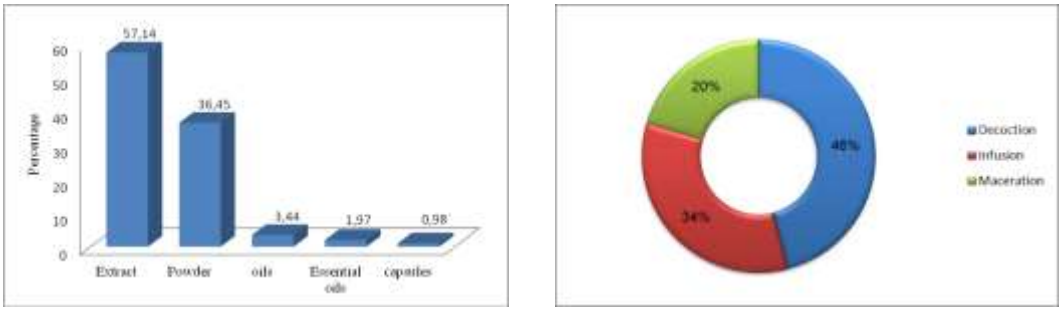

Figure 05: Percentage of preparation modes (A) and percentage of plant extraction methods of medicinal plants (B) used in the study area to treat liver diseases.

DISCUSSION

Traditional medicine is often a gender-specific practice, yet in certain cultures, both men and women engage in this activity[13]. Consequently, fewer female respondents were interviewed during the present study compared to male respondents. Ethnobotanical fieldwork is influenced by many aspects, including the cultural context of society, on-ground situations, the willingness of informants, and associated socio-cultural boundaries[14]. In the current study, (71%) of the respondents were aged 40-50 years or older, and the majority (90%) of respondents were literate. Therefore, there appears to be a relationship between the age of informants and the number of species reported, with older respondents reporting a larger number

of species. Younger people showed a lower interest in learning about and using ethnomedical practices, possibly due to their exposure to modern education. Simultaneously, the rapid progress in science and technology is leading the younger generation towards new traditions[15,16,26].

According to the findings of this study, residents in El-Oued state, Algeria, employ 78 different species of medicinal plants to treat liver disorders. These medicinal plants are divided into 41 families. The most important family was Asteraceae, with 14 species, followed by Apiaceae (7 species), Lamiaceae (7 species), and Brassicaceae (5 species). The other families are represented by various species, ranging from 1 to 5. This result illustrates the significant taxonomic variety of medicinal plants in the research region and the extensive information related to their use in conventional liver treatment10. Additionally, our study reports that various liver disorders can be treated with some plants more often than others. Zizyphus lotus (L.) Lam., Silybum marianum L., and Atriplex halimus L. were cited by 70% of the participants as sources of treatment for liver illnesses. This observation is consistent with several previous studies[17,18,19].

The results regarding growth forms of plant species revealed that 52% of plants were found spontaneously in the wild, while 48% were cultivated species. Similarly, research conducted by Slimani et al.,[20] (2016) in the Zerhoun region, Morocco, showed that the use of spontaneous medicinal plants is significantly higher (90%) than cultivated species (10%) in the treatment of various liver illnesses. This finding concerning growth forms (59% wild plants and 41% cultivated species) has also been reported by other authors[21]. Consequently, the levels of bioactive compounds in medicinal plants can vary based on several factors. These levels may be influenced by factors such as growth and development conditions, soil type, genotype, maturity, storage conditions, and extraction methods. The time and season of plant harvest can also affect its effectiveness[22].

Traditional healers use various parts of medicinal plants for medication. Among these plant parts, leaves are the most frequently utilized in the treatment of liver diseases, followed by seeds, roots, flowers, fruits, stems, and the entire plant. Our results align with the findings of Gebeyehu et al.,[23] (2014) and Belayneh et al.,[24] (2012). Furthermore, the highest accessibility of traditional drug formulations was observed when derived from the leaves of medicinal plant species, primarily due to the ease of collection and preparation, as well as the presence of bioactive substances in these plant parts. Similarly, leaves are considered the most potent and influential source for preparing traditional herbal medicine, as these plant parts play a vital role in the life cycle of plant species[8].

On the other hand, the results of the analysis regarding the condition of medicinal plant preparation, whether fresh or dry, indicated that the majority of medicinal plants were prepared from dried plant materials (77%), while 23% of medicinal plants were prepared from fresh plant material. These findings differ from those of several other studies[25,26,27].

Traditional medicine employs various preparation methods, including extraction, powder, oils, essential oils, capsules, and cataplasm. Users often seek the simplest ways to create herbal remedies. It is important to note that information regarding the use of medicinal plants and their therapeutic properties may vary from one person to another[28]. Based on the recorded data, we found that most interviewees used aerial parts such as leaves in the form of extracts (57.14%) and powders (36.45%). However, decoction (46%) and infusion (34%) were the most commonly used preparation methods. Several studies also report the prevalence of decoction as a preferred mode of preparation for medicinal[29,30]. According to researchers like Tahri et al.,[31] the use of decoction by the population is considered the best method to warm and disinfect the body.

Furthermore, Salhi et al.,[32] (2010) suggest that this

method can reduce toxicity when certain plants are combined. Some plants are used to create oils and ointments, especially for local applications. The consumption of fruits from certain plants, as well as the use of vegetable oil traditionally extracted from these fruits, were also noted[21].

CONCLUSION

This study has revealed that numerous medicinal plants in the El-Oued state of Algeria have the potential to treat various liver ailments. We documented a total of seventy-eight plant species from 41 different families during our research, with Asteraceae being the most prevalent family. Among these species, the leaves of plants are predominantly used to address liver-related issues. Additionally, decoction emerged as the most common traditional method of preparation in the region. The findings of this study underscore the continued use of traditional herbal remedies in El-Oued, despite the accessibility of modern healthcare and pharmaceuticals. Therefore, this research not only enriches local knowledge of medicinal flora but also establishes a valuable database for the exploration of new bioactive compounds with potential applications in pharmacology.

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الإمكانيات العلاجية للنباتات الطبية التقليدية من الجزائر في علاج أمراض الكبد

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ملخص

يتمثل هدف دراستنا في التحقيق العرقي النباتي للنباتات الطبية التقليدية التي يستخدمها الناس لعلاج أمراض الكبد في ولاية الوادي، جنوب شرق الجزائر. تم استخدام المقابلات والاستبيانات الشخصية لجمع البيانات. حيث أجريت مقابلات مع 156 مشارك، معظمهم في سن 50. سمحت دراسة النباتات الطبية لعلاج أمراض الكبد باكتشاف 78 نوعًا نباتي من 41 عائلة وأجناس نباتية مختلفة، بما في ذلك 52٪ من النباتات الطبية الموجودة في البرية. تم استخدام حوالي 77٪ من النباتات في المقام الأول في حالتها الجافة لتحضير العلاج. حيث كانت طريقة التغلية هي طريقة التحضير الأكثر استخدامًا، واستشهد 70٪ من المشاركين بـ *Zizyphus lotus* (L.) Lam. و *Silybum marianum* L. و *Atriplex halimus* L. كمصادر لعلاج أمراض الكبد. لقد كشف هذا التحقيق أن العديد من الأشخاص في منطقة البحث لا يزالون يعتمدون على العلاجات العشبية لعلاج اضطرابات الكبد. من ناحية أخرى، تقدم هذه الدراسة بيانات أساسية عن النباتات الطبية والتي ستكون بمثابة نقطة انطلاق للبحوث المستقبلية المكثفة.

الكلمات الدالة: علم النبات العرقي، الأدوية، النباتات الطبية، امراض الكبد، الوادي.

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Decriminalization of Narcotics in Jordanian Legislation: Theory and Practice

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ABSTRACT

This study delves into recent legislation in Jordan regarding the decriminalization of narcotics and traces the evolution of related legislation in the country. It explores the definition and underlying philosophy of decriminalization while examining arguments from both proponents and opponents of drug decriminalization from a jurisprudential perspective. Additionally, the research sheds light on practices that have emerged in response to the decriminalization of narcotics in other jurisdictions. The study thoroughly examines the advantages and disadvantages of decriminalizing narcotics, analyzing its potential impact on drug consumption. Finally, the researcher proposes the implementation of a gradual and partial systematic plan within Jordanian legislation to address the growing trend of drug decriminalization. The paper also provides insights into the stances of the United States and other countries on this issue and how their legislations have addressed it.

Keywords: Decriminalization of Narcotics, Drug Legalization, Positivism philosophy, Natural drugs, Synthetic drugs, Drug consumption.

1. INTRODUCTION

Psychoactive medications are compounds that alter mental processes such as perception, consciousness, cognition, mood, and emotions when consumed or introduced into the body[1]. These medicines belong to a broader category of psychoactive substances, which also includes alcohol and nicotine[2]. The term "psychoactive" may not always imply the potential for dependence, and in everyday language, it is often used without further explanation, as seen in phrases like "drug use," "substance use," or "substance abuse"[3]. In 1946, the Economic and Social Council (ECOSOC) passed resolution 9(I), establishing the Commission on Narcotic Drugs (CND) to assist ECOSOC in monitoring global drug control agreements. Later, in 1991, the General Assembly (GA) granted the CND the authority to oversee UNODC. The CND's agenda comprises a normative section for treaty-based and normative functions and an operational section for UNODC governance[4].

The notion that whatever is prohibited becomes desirable is applicable to various thoughts and ideas, including drug usage. Many countries have adopted punitive measures against drug consumption, trade, and possession, categorizing drug users as criminals[5]. Conversely, there are countries that consider drug users as victims in need of treatment rather than punishment[5]. Consequently, the focus should be on providing treatment

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and support rather than incarceration[6]. Criminalizing certain drugs can have several adverse consequences [6]. One of the most significant impacts is the empowerment of the illicit drug trade, making it difficult to regulate and control[7].

In a Jordanian study involving cannabis and alcohol addicts, 19 participants used alcohol, while 26 used hashish. The researcher employed the Family Socialization Scale and the Attitudes Toward Drug and Narcotics Abuse Measure to achieve the study's objectives. The study revealed that addiction had the highest mean among participants, with statistically significant correlation coefficients between family socialization and addiction. Addiction also demonstrated a strong association with both authoritarian and lenient socialization patterns, and a negative relationship with democracy. Furthermore, the results indicated that family socialization patterns could predict 72% of addiction, with an R2 value of 0.520, explaining 49% of the variance [8].

There is an ongoing debate regarding drug decriminalization, with some arguing that only drug traders should face penalties, while others advocate for penalizing drug users as well, considering them criminals. This research argues against criminalizing drug consumers in Jordan and instead proposes government regulation to mitigate potential adverse consequences.

The research presented in this paper is structured into three main sections. The first section introduces the concept of drug decriminalization and its underlying philosophy. The second section delves into the jurisprudential controversy surrounding decriminalization, exploring various commentators' perspectives. Lastly, the third section examines different legislations from various countries, particularly comparing the United States to Jordanian legislation.

According to the positivist philosophy in criminology, drug addiction should be approached from a medical perspective. This perspective views drug users as individuals in need of medical attention rather than as criminals. While it acknowledges that some users may engage in criminal activities to sustain their drug supply, it deems it unjust to classify them solely as criminals due to their drug use[9].

To address the issue of narcotic drugs, the New York Academy of Medicine proposed a strategy in which addicts would receive narcotics at a reduced cost under government supervision while undergoing withdrawal therapy. This approach aims to treat addicts as patients rather than criminals, ensuring they seek proper medical treatment and monitoring. By providing legal access to narcotic medications at minimal cost under strict medical supervision, the strategy aims to eliminate the need for criminal activity to obtain opioids and reduce the motivation to create new addicts. Consequently, it could lead to the disappearance of black markets associated with narcotics. This alternative approach would place strict control over narcotics on the side of law and order rather than against it[10].

The philosophy of drug decriminalization is rooted in the notion that, while drug use can be harmful, it doesn't necessarily constitute an absolute evil warranting the high levels of imprisonment often associated with national "war on drugs" policies. Proponents argue that punitive measures lack a logical rationale and are disproportionate to an action that, while potentially unhealthy for the user, is typically not directly harmful or antagonistic to society. They contend that making drugs legal would prevent law-abiding businesses from being sidelined by illegal drug trade. This paper asserts that decriminalization serves to deter illicit traders from capitalizing on this lucrative market, as the added cost of prohibition can significantly reduce their profits[11].

Decriminalizing drugs can mitigate serious health risks from a hygienic standpoint, as it facilitates formal health supervision of drug production, distribution, and use, including safe injection practices. Instead of a blanket ban, the justice system should focus on regulation. Statistics reveal that around 25% of AIDS cases in Washington,

D.C., in the USA, before 1990, were linked to the lack of access to clean needles for drug users. The prohibition of drugs, in contrast to alcohol, represents a legal inconsistency in the USA[12,13].

It's important to recognize that not every opioid transforms its user into a deranged, violent individual capable of committing heinous crimes. In fact, many drugs induce lethargy rather than aggression. A nationwide legalization of marijuana in the United States could significantly reduce drug trafficking groups' earnings from drug exports, potentially by one-fifth to one-third. Consequently, banning narcotics while permitting alcohol raises legal contradictions.

The most compelling counterargument against drug decriminalization is the concern that legal access to drugs may lead more individuals, both experienced users and newcomers, to experiment with substances because they are no longer prohibited. This, in turn, could lead to increased addiction rates, as users no longer face criminal charges for drug possession or use. It appears that when something is legalized, it may become more accessible to those who desire it[12].

Opponents argue that narcotics have a very elastic demand, meaning that if drugs were legalized and their prices decreased, the quantity purchased would increase significantly[14].

Many are against the decriminalization of narcotics due to their strong association with criminal activities. They believe that legalizing drugs would lead to an increase in crime rates. Research has shown that nearly half of the homicides in the United States are somehow linked to alcohol and/or drugs[10]. Furthermore, decriminalization alone would not eliminate the thriving underground market for marijuana, estimated to be worth $40 billion or more in the U.S.[15].

Why is decriminalization considered insufficient? Despite its advantages, decriminalization falls short in many respects, primarily because it still operates within the framework of prohibition. Consequently, it continues to suffer from the inherent issues of prohibition, including an illegal and unregulated market, unequal enforcement of laws (regardless of the severity of the penalty) against specific groups, particularly people of color, and the presence of unregulated products with unknown potency and quality[16,17].

In Portugal, prior to the passing of Law No. 30/2000, there were concerns and significant criticism regarding drug decriminalization, especially from right-wing politicians, traditional societal sectors, and certain mass media outlets. Some argued that it might lead to a sudden increase in drug use and turn Portugal into a drug paradise, attracting drug tourism and foreigners who could use drugs without the risk of serious legal consequences[11]. This research aims to investigate drug decriminalization in the context of reducing consumption in Jordan and draw comparisons with different jurisdictions such as the USA.

2. METHODOLOGY

This study provides a comprehensive review of the Convention on Narcotic Drugs and Psychotropic Substances, considering the viewpoints of both proponents and opponents of drug decriminalization. It emphasizes that the effectiveness of narcotic drug decriminalization is more pronounced at the international level rather than solely at the national level. Additionally, the study assesses the influence and patterns of comparative drug decriminalization legislation on the extent of narcotic drug consumption.

Furthermore, it is proposed that Jordanian legislation adopts a systematic and gradual approach to address the ongoing trend of drug decriminalization. This research also explores the impact of decriminalizing narcotic substances on the illicit drug usage landscape.

3. RESULTS AND DISCUSSION:

3.1. Analysis of the impact of decriminalization of Narcotic drugs on the magnitude of drug consumption:

One significant reason for decriminalization is that

when drugs are readily available, consumers may no longer desire excessive quantities due to diminishing marginal utility. This can lead to a reduction in the drug market and eventually bring an end to the fruitless war on drugs[12].

The argument that decriminalization could lead to a significant increase in drug use and a fall in prices can be countered as follows[14]:

1- Narcotics are considered necessities for drug users, not luxuries, making them less responsive to price increases. There is no compelling reason to assume that many people will suddenly begin using drugs solely because it is allowed.

2- Legalization or decriminalization does not negate the recognition of the dangers associated with drugs.

3- Legalization or decriminalization can lead to a decrease in drug potency. With a regulated supply, people may opt for weaker and safer drugs. The combination of a regulated supply and the low elasticity of demand for drugs can help maintain consumption at a reasonable level.

The concern that legalizing drugs may not eliminate lucrative underground markets is based on the assumption that black market transactions would continue in secret to evade government price controls and taxes. It's important to note that during the Prohibition Era in the United States, alcohol was prohibited, leading to a thriving black market. However, once alcohol was legalized, the need for secrecy diminished. While wholesalers eventually faced taxes, the alcohol industry became less violent and dangerous. A similar principle could be applied to the pharmaceutical industry[12].

3.2. Trends of Comparative Legislation:

Since 2011, hemp and its derivatives containing less than 1% THC have not been classified as narcotics under Swiss legislation. However, the so-called "light cannabis" boom began in 2017 when it became legal in Switzerland to cultivate and sell unprocessed inflorescences of this plant as long as the THC level was less than 1%[18].

I In 2001, Portugal decriminalized narcotics (Law no. 30/2000), resulting in significant reductions in overdose, addiction, and infection rates caused by contaminated needles. Statistics show that among Portuguese adults, there are four drug overdose deaths for every million citizens [19], compared to the European Union average of 14.8 per million in 2019[18]. In contrast, overdose fatalities in the United States exceeded 72,000 in 2017. If the overdose mortality rates in the United States were similar to those in Portugal, there would have been fewer than 800 overdose fatalities in that year[20].

The threshold amounts of prohibited drugs considered as personal possession are specified in Portuguese Law No. 30/2000. These levels primarily serve as a starting point for prosecutors to evaluate whether the person's possession of the substance is for personal use, which is decriminalized, or for trafficking, which may result in a jail sentence of one to 12 years. Drug trafficking penalties may be as high as 25 years in "aggravating circumstances," which include trafficking as part of a criminal organization and causing death or severe bodily injury[21].

Several other nations, including the Czech Republic, Spain, and the Netherlands, have had successful experiences with decriminalization. Additionally, countries like Canada, France, Georgia, Ghana, Ireland, and Norway are now exploring methods to abolish the criminality of personal drug use[21].

In the United States, a few states, such as Colorado, Virginia, and Washington[22], have legalized natural marijuana for recreational use. For example, Colorado legalized recreational marijuana under Amendment 64, allowing personal use and growth within certain limits. Anyone aged 21 and over can legally possess up to an ounce of marijuana, which can be purchased at licensed stores[19].

Furthermore, some countries have legalized the trade of marijuana. Uruguay became the first country in the world to legalize and regulate the marijuana trade in 2013, and Canada became the second country to do so, passing marijuana legalization in 2017 and implementing

legislation to permit a national marijuana market. Additional legalization proposals are under consideration in several other countries[23].

Nevertheless, decriminalizing narcotic drugs only at the national level may hinder the goal of effectively controlling the drug market. It is argued in this paper that decriminalizing drugs in isolation from other countries, particularly neighboring countries like those in the Middle East, would be unnecessary. This is because it could create imbalanced conditions for the flow of drugs across borders, making drug control and regulation challenging. In contrast to policies aimed at reducing narcotics, this approach assumes that reducing narcotics shipments is influenced by disparities in drug policies. Therefore, the current Convention on Narcotic Drugs and Psychotropic Substances needs to be reviewed from the perspectives of both opponents and proponents of decriminalization.

3.3. **The trend of Jordanian legislation concerning the decriminalization of Narcotics:**

Jordan participated in the United Nations conference to approve the Single Convention on Narcotic Drugs at the United Nations headquarters in 1961. Furthermore, Jordan signed its final document and the protocol of amendments to this Convention in 1972. Jordan also ratified the 1988 United Nations Convention against Illicit Traffic in Narcotic Drugs and Psychotropic Substances[24,25].

Jordan continues to fulfill its international obligation to combat illegal drugs and psychoactive substances through national law. Jordan's Narcotics and Psychotropics Act No. 23 of 2016 and its subsequent amendments contain legal provisions that criminalize related actions, including the use and possession of narcotic and psychotropic drugs.

In 2020, there were 16,118 cases of narcotics drug abuse and possession in Jordan, while drug trafficking cases for the same year numbered 3,937. The drug crime rate per 10,000 population for 2020 was 19, indicating an increase since 2011, as depicted in Figure 1 below[25].

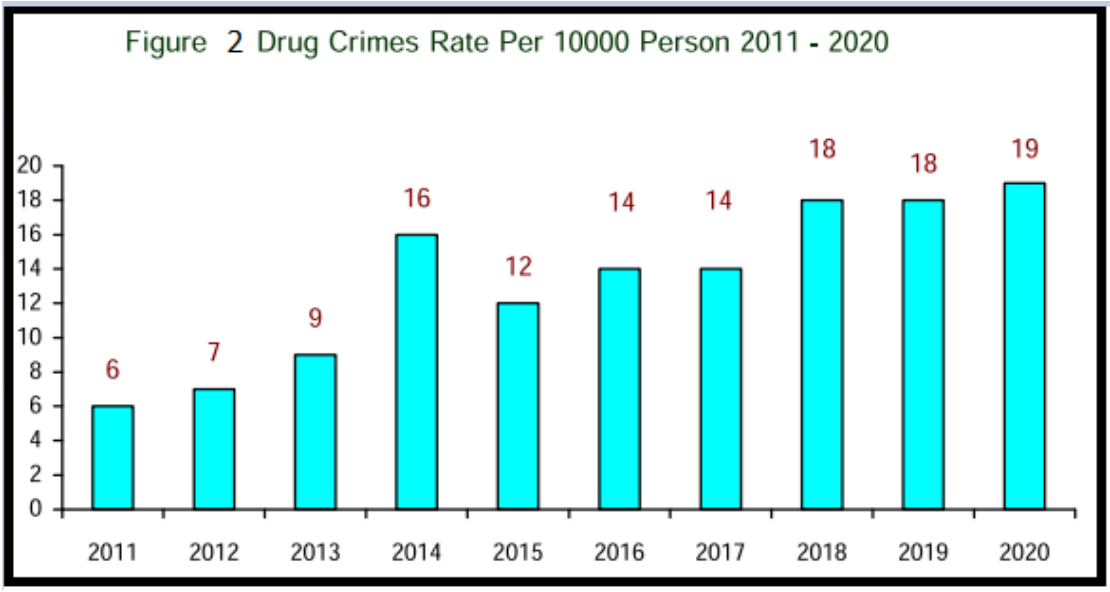

Figure 1: Drug crimes rate per 10000 persons 2011-2020 in Jordan

While full decriminalization of narcotic drugs in Jordan is not possible due to Jordan's commitment to the 1988 United Nations Convention against Illicit Traffic in Narcotic Drugs and Psychotropic Substances, which prohibits the legalization or decriminalization of all types of illicit narcotics and psychotropic substances, partial decriminalization of narcotics in Jordan has been achieved. The first indications of this appeared in the repealed Act No. 11 on Narcotic Drugs and Psychotropic Substances of 1988, which was amended in 2013. In this amended law, Section 14 for the first time prohibited the prosecution of offenders involved in narcotic drugs and psychotropic substances, stipulating that they would be transferred for treatment to a specialized narcotics control center or another hospital approved by the Ministry of the Interior, and this action would not be considered a recurrence.

This unique approach to decriminalization of drug abuse has sparked a contentious debate in Jordan. Opponents viewed it as potentially encouraging young people to try drugs. However, this exceptional provision of the law has since been revised in the current Law No. 23 of 2016. Article 9 of this law criminalizes all activities related to substances listed in the annex tables of the law, including abuse and possession with intent to abuse. Article 14 criminalizes all activities related to substances not listed in the annex tables of the law with intent to cause anesthesia or any other harmful effect on the mind. However, a first-time offense is still not counted as recidivism (Article (9)/b).

Article 9 (c) of the current Law on Narcotics and Psychotropic Substances (No. 23 of 2016 and its amendments) allows the judge to replace imprisonment for perpetrators in cases involving the consumption, acquisition, smuggling, import, export, possession, obtaining, purchase, receipt, transportation, production, manufacture, storage, or cultivation of narcotics, psychotropic substances, preparations, or plants intended for abuse. The judge may opt for alternative procedures as deemed appropriate for the individual's case, including:

1 .Admitting the perpetrator to a specialized clinic for drug rehabilitation for a duration determined by a special medical committee.

2. Admitting the perpetrator to a specialized clinic for psychological and social treatment for narcotics addiction, under the supervision of a psychiatrist or social specialist.

Recognizing that Jordanian legislation views narcotic drug abusers as individuals in need of medical help rather than criminals, the current law continues to prevent the prosecution of individuals who abuse narcotic drugs and psychotropic substances or are addicted to them if they voluntarily seek treatment before arrest (Article (9)/f).

However, this paper contends that the stance of the current Jordanian Law No. 23 on Narcotic Drugs and Psychotropic Substances of 2016 and its amendments is insufficient to address the increasing issue of narcotic drug abuse. This law should encourage first-time abusers and possessors to seek assistance rather than hide to avoid legal consequences. Perpetrators of drug abuse and possession require rehabilitation, not punishment. The current law policy incentivizes them to conceal their accidental abuse or possession instead of seeking help. This paper proposes decriminalizing accidental abuse and possession for the first time in Jordanian law, as was the case in the 2013 amendment to the repealed Narcotics and Psychotropic Substances Law.

4. CONCLUSIONS

In the Middle East, decriminalizing drugs in isolation from other countries, particularly neighboring countries such as those in the Middle East, would be unnecessary. This is because it would create imbalanced conditions for the flow of drugs across borders, rendering drug control and regulation ineffective. Anti-narcotic drug legislation should be reconsidered from the perspectives of both opponents and proponents of decriminalization. On an international level, there is a need to review the Convention on Narcotic Drugs and Psychotropic Substances. Decriminalizing accidental drug abuse and possession for the first time in Jordan would represent a significant step in the right direction.

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عدم تجريم المخدرات في التشريع الأردني: النظرية والممارسة

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ملخص

تسلط هذه الدراسة الضوء على عدم تجريم المخدرات في التشريعات الحديثة في الأردن وتطور التشريعات حول هذا الموضوع، كما تنظر الدراسة في تعريف وفلسفة نزع التجريم وتبحث في كل من مؤيدي ومعارضي منع تجريم المخدرات من وجهة نظر الفقه، كما تبرز هذه الدراسة بعض الممارسات التي ظهرت نتيجة لعدم تجريم المخدرات في بعض الولايات القضائية الخارجية. نوقشت في هذا البحث فوائد ومساوئ عدم تجريم المخدرات، وتم تحليل أثر عدم تجريم المخدرات على استهلاك المخدرات، وأخيراً يقترح البحث أن تنفذ التشريعات الأردنية خطة منهجية تدريجية وجزئية لمعالجة اتجاه منع تجريم المخدرات، وتسلط الورقة الضوء على مواقف الولايات المتحدة الأمريكية وغيرها من البلدان بشأن هذه المسألة وكيفية معالجة تشريعاتها لها.

الكلمات الدالة: عدم تجريم المخدرات، تقنين المخدرات، فلسفة الوضعية، الأدوية الطبيعية، العقاقير الاصطناعية، استهلاك المخدرات .

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Exploration of Potentially Bioactive Compounds from Fingerroot (*Boesenbergia rotunda* L.) as Inhibitor of Atherosclerosis-Related Proteins (CETP, ACAT1, OSC, sPLA2): An *in silico* Study

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ABSTRACT

Boesenbergia rotunda L., commonly known as fingerroot, is recognized as one of Indonesia's medicinal plants with significant potential for treating various diseases, including atherosclerosis. This study aims to analyze the anti-atherosclerosis potential of bioactive compounds found in fingerroot by assessing their inhibitory effects on four proteins associated with atherosclerosis (CETP, ACAT1, OSC, and sPLA2). Bioactive compounds from B. rotunda were retrieved from the KnapSack database. The drug-likeness properties were predicted using the SwissADME web server, and the bioactivity of the compounds was assessed using the PASSOnline server. The identification of active sites on proteins and the validation of protein structures were performed using the SCFBio web server and Autodock Vina. Specific docking simulations between fingerroot compounds and the target proteins were carried out using AutoDock Vina. The analysis revealed that fingerroot contains 20 bioactive compounds with favorable drug-like properties. Among these, dihydrochrysin, sakuranetin, isopimaric acid, 2S-pinocembrin, 5,7-dihydroxy-8-C-geranylflavanone, 7,4'-dihydroxy-5-methoxyflavanone, and 5,7-dihydroxy-8,7-methoxy-5-hydroxy-8-geranylflavanone were predicted to exhibit anti-atherosclerosis activities. In the interactions with CETP, rubranine and (-)-4-hydroxypanduratin A showed the lowest binding affinity scores. Meanwhile, in interactions with ACAT1, OSC, and sPLA2, rubranine and 5,7-dihydroxy-8-C-geranylflavanone displayed the lowest binding affinities. In conclusion, fingerroot exhibits high potential as an anti-atherosclerosis agent through the inhibition of four proteins associated with atherosclerosis, as predicted through in silico analysis.

Keywords: ACAT1, atherosclerosis, CETP, molecular docking, OSC, sPLA2e.

INTRODUCTION

Atherosclerosis is an inflammatory disease initiated by the accumulation of lipids in the vessel wall, leading to vascular narrowing or blockage and disrupting blood flow[1]. According to WHO data from 2016, approximately 17.9 million people worldwide died from cardiovascular diseases, which were identified as the leading cause of death globally[2]. In Indonesia, one-third of all deaths are attributed to cardiovascular diseases, including

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atherosclerosis[3]. Several proteins play critical roles in the formation of atherosclerosis[4].

Proteins such as CETP, ACAT1, OSC, and sPLA2 have been implicated in atherosclerosis. Cholesteryl ester transfer protein (CETP) is responsible for transporting and converting cholesterol esters into LDL, IDL, and VLDL, which lowers HDL levels and raises LDL levels[5]. Acyl-CoA:cholesterol acyltransferase (ACAT1) is a protein involved in the re-esterification of cholesterol absorbed by macrophages, leading to the formation of foam cells[6]. Oxido-squalene-cyclase (lanosterol synthase, OSC) is the enzyme responsible for the cholesterol synthesis pathway[7]. Meanwhile, sPLA2 is involved in modifying lipoproteins, producing products that can induce inflammation and initiate the formation of atherosclerotic plaques[8]. Inhibiting these proteins could suppress the development of atherosclerosis[4]. Standard drugs used to treat cardiovascular diseases, such as statins, carry risks such as statin-associated muscle symptoms (SAMS), myopathy, and diabetes[9]. Herbal medicine is an alternative way to treat diseases.

Boesenbergia rotunda (fingerroot), a member of the Zingiberaceae family, is known as one of Indonesia's medicinal plants. Fingerroot has been used to treat gastrointestinal ailments, muscle pain, rheumatism, dyspepsia, inflammatory conditions like swelling and dermatitis, dysentery, diuretic, and diarrhea. Compounds found in fingerroot have reported antimicrobial, antiparasitic, anti-scabies, anti-cancer, antioxidant, and anti-inflammatory properties[10,13]. Through in silico analysis, one can assess the physicochemical and pharmacokinetic properties of drug candidates, thereby improving the quality of the drug development process[14]. This study aims to analyze the anti-atherosclerosis potential of bioactive compounds in fingerroot by examining their inhibitory effects on four proteins involved in atherosclerosis development (CETP, ACAT1, OSC, and sPLA2).

Experimental Section

Data Retrieval

The list of bioactive compounds from fingerroot (B. rotunda L.) was obtained from the KnapSack database (http://www.knapsackfamily.com/KNApSAcK/). The compound names, formulas, PubChem IDs, and SMILES representations are presented in Table 2.

Drug-likeness Prediction

In this study, drug-likeness prediction was conducted using the SwissADME web server (http://www.swissadme.ch/)[14,15]. The prediction results were selected based on Lipinski, Veber, and Egan rules. Various parameters were considered, including molecular weight, MlogP value, number of hydrogen bond acceptors (nON), number of hydrogen bond donors (nOHNH), total number of rotatable bonds, and total polar surface area (TPSA).

Bioactivity Prediction

Bioactivity prediction of the compounds was conducted using the PASSOnline web server (http://way2drug.com/PassOnline/). Several parameters related to atherosclerosis, such as cholesterol synthesis inhibition, anti-hypercholesterolemia, anti-inflammatory, and antioxidant properties, were taken into account[16–19]. Compounds with a probability of activity (Pa) greater than 0.7 are considered to have high pharmaceutical potential, while those with Pa values between 0.5 and 0.7 are considered to have low pharmaceutical potential[20].

Protein Active Site Prediction and Validation

Protein active site prediction aimed to predict the location of the active site of four proteins. Active site prediction performed using SCFBio web server (http://www.scfbio-iitd.res.in/dock/ActiveSite.jsp). The active site prediction is validated by blind docking between the protein and the reference inhibitor drug. Several inhibitors were used, such as anacetrapib

(11556427) for CETP, ART-101 (131679) for ACAT1, Ro 48-8071 (9853053) for OSC, and KH064 (164754) for sPLA2. The inhibitor which binds to the SCFBio predicted site was strongly predicted as the potential active site.

Molecular Docking Simulation

Proteins, including cholesteryl ester transfer protein (CETP), acyl-CoA:cholesterol acyltransferase (ACAT1), oxidosqualene cyclase (OSC), and acidic secretory phospholipase A2 (sPLA2), were prepared by removing contaminant molecules using Biovia Discovery Studio 2019 software (Dassault Systèmes Biovia, San Diego, California, USA). All compounds were subjected to energy minimization using the Open Babel tool integrated into the PyRx software. Specific docking was performed using the AutoDock Vina software, which is integrated into PyRx[21]. The grid positions were set at the active site of each protein (Table 1). The docking results were visualized using Biovia Discovery Studio 2019 software[22].

Tabel 1. Grid settings for specific docking

Proteins	PDB ID	Grid position					
		Center			Dimensions		
		X	Y	Z	X	Y	Z
CETP	4ews	12.7646	-3.2357	45.2502	25.000	25.4289	31.2910
ACAT1	6p2p	97.5383	154.6754	162.1431	30.9911	37.1683	35.1102
OSC	1w6k	42.2596	54.8271	27.1112	36.1945	43.1945	30.6761
sPLA2	1dcy	60.4890	29.4733	43.8285	16.3735	24.9770	22.1178

RESULTS AND DISCUSSION

Compounds Contained in Fingerroot

According to KnapSack, the majority of bioactive compounds in fingerroot belong to the flavonoid group, with some essential oils. Fingerroot contains a total of 14 flavonoid compounds. In our study, only one essential oil, E-geraniol, was identified in fingerroot. Additionally, fingerroot contains cylohexane derivatives such as (+)-Zeylenol and Crotepoxide. Another compound present in fingerroot is 2,4-dihydroxy-6-phenethyl-benzoic acid methyl ester (Table 2). The flavonoid group is the most abundant bioactive compound category found in fingerroot rhizomes, consisting of chalcones, flavones, and flavanones. Chalcones compounds include cardomonin and flavokawin A. Furthermore, perylanated chalcones such as boesenbergia A, rubranine, (-)-4-hydroxypanduratin A, and isopanduratin A are also present. The flavanones category includes compounds like sakuranetin, alpinetin, 5,7-dihydroxy-8-C-geranylflavanone, 7,4'-dihydroxy-5-methoxyflavanone, and 7-methoxy-5-hydroxy-8-geranylflavanone (Table 2).

Table 2. Compounds in Fingerroot obtained from KnapSack

Compounds	Formula	Pubchem ID	SMILES
(E)-geraniol[b]	$C_{10}H_{18}O$	637566	CC(=CCCC(=CCO)C)C
Dihydrochrysin[a]	$C_{15}H_{12}O_4$	238782	C1C(OC2=CC(=CC(=C2C1=O)O)O)C3=CC=CC=C3
Sakuranetin[a]	$C_{16}H_{14}O_5$	73571	COC1=CC(=C2C(=O)CC(OC2=C1)C3=CC=C(C=C3)O)O
Isopimaric acid[c]	$C_{20}H_{30}O_2$	442048	CC1(CCC2C(=CCC3C2(CCCC3(C)C(=O)O)C)C1)C=C
Cardamomin[a]	$C_{16}H_{14}O_4$	641785	COC1=CC(=CC(=C1C(=O)C=CC2=CC=CC=C2)O)O
Flavokawin A[a]	$C_{18}H_{18}O_5$	270057	COC1=CC=C(C=C1)CCC(=O)C2=C(C=C(C=C2OC)OC)O
Boesenbergin A[a]	$C_{26}H_{28}O_4$	6313827	CC(=CCCC1(C=CC2=C(C=C(C(=C2O1)C(=O)C=CC3=CC=CC=C3)O)OC)C)C
Rubranine[a]	$C_{25}H_{26}O_4$	42607681	CC1(C2CCC3(CC2C4=C(O3)C=C(C(=C4O1)C(=O)C=CC5=CC=CC=C5)O)C)C
Panduratin A[a]	$C_{26}H_{30}O_4$	6483648	CC1=CCC(C(C1CC=C(C)C)C(=O)C2=C(C=C(C=C2O)OC)O)C3=CC=CC=C3
Alpinetin[a]	$C_{16}H_{14}O_4$	154279	COC1=CC(=CC2=C1C(=O)CC(O2)C3=CC=CC=C3)O
5,7-Dihydroxy-8-C-geranylflavanone[a]	$C_{25}H_{28}O_4$	11143678	CC(=CCCC(=CCC1=C2C(=C(C=C1O)O)C(=O)CC(O2)C3=CC=CC=C3)C)C
7,4'-Dihydroxy-5-methoxyflavanonea	$C_{16}H_{14}O_5$	188424	COC1=CC(=CC2=C1C(=O)CC(O2)C3=CC=C(C=C3)O)O
(-)-4-Hydroxypanduratin A[a]	$C_{25}H_{28}O_4$	636530	CC1=CCC(C(C1CC=C(C)C)C(=O)C2=C(C=C(C=C2O)O)O)C3=CC=CC=C3
Isopanduratin A[a]	$C_{26}H_{30}O_4$	10069916	CC1=CCC(C(C1CC=C(C)C)C(=O)C2=C(C=C(C=C2OC)O)O)C3=CC=CC=C3
2,4-Dihydroxy-6-phenethyl-benzoic acid methyl ester[e]	$C_{16}H_{16}O_4$	14195786	CC1=CCC(C(C1CC=C(C)C)C(=O)C2=C(C=C(C=C2O)O)O)C3=CC=CC=C3
5,6-Dehydrokawain[a]	$C_{14}H_{12}O_3$	5273621	CC1=CCC(C(C1CC=C(C)C)C2=CC=CC=C2)C(=O)C3=C(C=C(C=C3OC)O)O
7-Methoxy-5-hydroxy-8-geranylflavanone[a]	$C_{26}H_{30}O_4$	129864052	COC(=O)C1=C(C=C(C=C1O)O)CCC2=CC=CC=C2
(+)-Zeylenol[d]	$C_{21}H_{20}O_7$	14283260	COC1=CC(=O)OC(=C1)C=CC2=CC=CC=C2
Crotepoxide[d]	$C_{18}H_{18}O_8$	161314	C1=CC=C(C=C1)C(=O)OCC2(C(C=CC(C2O)OC(=O)C3=CC=CC=C3)O)O

[a]Flavonoid group, [b]Essential oil, [c]rosin compounds, [d]cyclohexane derivatives, [e]other compounds

Drug-likeness Prediction

Drug-likeness prediction involves assessing the potential of compounds to become drug candidates based on factors such as chemical structure stability, solubility, and permeability. Most of the active compounds found in fingerroot exhibit favorable bioavailability as oral drugs, indicated by the satisfaction of the rule of Lipinski[23], Veber[24], and Egan[25] (Table 3). However, some compounds, including isopimaric acid, boesenbergin A, panduratin A, isopanduratin A, and 7-methoxy-5-hydroxy-8-geranylflavanone, violate specific criteria within these rules. For instance, isopimaric acid has an MlogP value exceeding 4.15, leading to a violation of one of Lipinski's rules. Compounds that violate two or more of the 'Lipinski Rule of Five' criteria are typically considered to have low druggability[26]. On the other hand, boesenbergin A, panduratin A, isopanduratin A, and 7-methoxy-5-hydroxy-8-geranylflavanone violate Egan's criteria for lipophilicity due to their WlogP values exceeding 5.88. When the WlogP value surpasses 5, it indicates high lipophilicity or low solubility, potentially affecting the compound's absorption within the body[27].

Table 3. Druglikeness prediction result

Compounds	Druglikeness parameters							Violation		
	MW (g/mol)	MlogP	nON	nOHNH	WlogP	RB	TPSA (Å²)	L[a]	V[b]	E[c]
(E)-geraniol	154.25	2.59	1	1	2.67	4	20.23	**0**	**0**	**0**
Dihydrochrysin	256.25	1.27	4	2	2.48	1	66.76	**0**	**0**	**0**
Sakuranetin	286.28	0.96	5	2	2.49	2	75.99	**0**	**0**	**0**
Isopimaric acid	302.45	4.54	2	1	5.21	2	37.30	**1**	**0**	**0**
Cardamomin	270.28	1.83	4	2	2.89	4	66.76	**0**	**0**	**0**
Flavokawin A	316.35	1.83	5	1	3.23	7	64.99	**0**	**0**	**0**
Boesenbergin A	404.50	3.51	4	1	5.99	7	55.76	**0**	**0**	**1**
Rubranine	390.47	3.46	4	1	5.39	3	55.76	**0**	**0**	**0**
Panduratin A	406.51	3.59	4	2	6.01	6	66.76	**0**	**0**	**1**
Alpinetin	270.28	1.52	4	1	2.78	2	55.76	**0**	**0**	**0**
5,7-Dihydroxy-8-C-geranylflavanone	392.49	3.38	4	2	5.72	6	66.76	**0**	**0**	**0**
7,4'-Dihydroxy-5-methoxyflavanone	286.28	0.96	5	2	2.49	2	75.99	**0**	**0**	**0**
(-)-4-Hydroxypanduratin A	392.49	3.38	4	3	5.71	5	77.76	**0**	**0**	**0**
Isopanduratin A	406.51	3.59	4	2	6.01	6	66.76	**0**	**0**	**1**
2,4-Dihydroxy-6-phenethyl-benzoic acid methyl ester	272.30	2.72	4	2	2.67	5	66.76	**0**	**0**	**0**
5,6-Dehydrokawain	228.24	2.06	3	0	2.6	3	39.44	**0**	**0**	**0**
7-Methoxy-5-hydroxy-8-geranylflavanone	406.51	3.59	4	1	6.02	7	55.76	**0**	**0**	**1**
(+)-Zeylenol	384.38	1.44	7	3	1.09	7	113.29	**0**	**0**	**0**
Crotepoxide	362.33	0.75	8	0	0.63	8	103.96	**0**	**0**	**0**

[a]L = Lipinsky: MW≤500, MlogP≤4.15, nON≤10, nOHNH≤5, [b]V = Veber: RB≤10, TPSA≤140, [c]E = Egan: WlogP≤5.88, TPSA≤131.6

Compounds Bioactivity Prediction

The PASS Online prediction results indicate that 5,7-dihydroxy-8-c-geranylflavanone and 7-methoxy-5-hydroxy-8-geranylflavanone are bioactive compounds with the highest cholesterol synthesis inhibitor activity among all the compounds found in fingerroot. These compounds also

exhibit a high potential as anti-inflammatories (Fig. 1). In our study, isopimaric acid was identified as having a high potential for both anti-inflammatory and anti-hypercholesterolemia activities (Fig. 1). Additionally, PASS Online prediction identified six compounds with Pa > 0.7 values, signifying a high potential for anti-hypercholesterolemia activity. These compounds include dihydrochrysin, sakuranetin, 7,4'-dihydroxy-5-methoxyflavanone, isopimaric acid, and 7-methoxy-5-hydroxy-8-geranylflavanone. Furthermore, dihydrochrysin, 7,4'-dihydroxy-5-methoxyflavanone, 5,7-dihydroxy-8-c-geranylflavanone, and 7-methoxy-5-hydroxy-8-geranylflavanone exhibit the highest antioxidant activity with Pa > 0.7 values (Fig. 1). Previous research has suggested that sakuranetin can reduce inflammation in a rat asthma model[28]. Isopimaric acid was found in *C. Japonica* has antioxidant and anti-inflammatory activity[29].

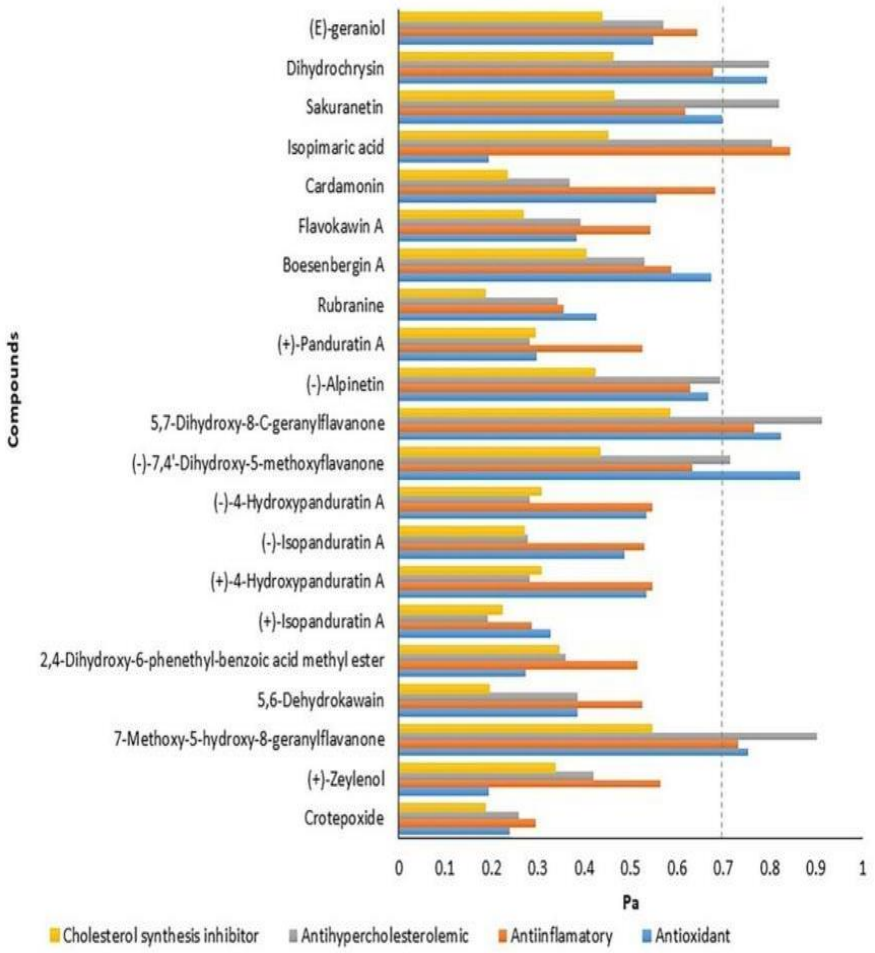

Figure 1. Bioactivity prediction of compounds contained in fingerroot.

Protein Active Site

The results of active site prediction indicated that nearly all the active sites of the four proteins were located within regions of high hydrophobicity (Fig. 2). The active site of CETP is situated around residues Ile15-Leu471 and exhibits high hydrophobicity. Similarly, the active site of ACAT1 is positioned around Glu170-Gln521 and displays a high level of hydrophobicity. In contrast, the active site of OSC is situated around the amino acid Phe74-Trp590 and has a lower degree of hydrophobicity. The active site of sPLA2 is located around the amino acid Asn1-Ser65 and demonstrates high hydrophobicity.

The results of blind docking simulations between the four proteins and their inhibitors confirmed that all inhibitors bound to the predicted active site of the respective protein, thereby reinforcing the accuracy of the active site predictions. The active site of a protein plays a crucial role in its overall activity, as it is involved in catalysis, substrate binding, and stabilizing the reactions occurring within the protein's cavity[30]. The protein's active site consists of residues that are important for carrying out binding and catalytic functions[31]. One effective strategy to inhibit protein activity is to block the protein's active site using competitive[32].

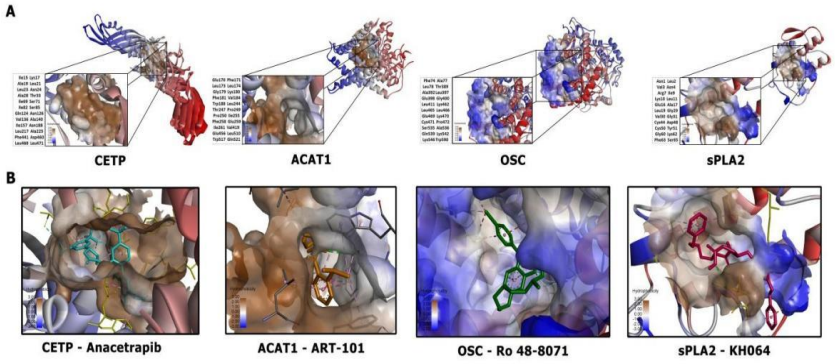

Figure 2. Active site prediction and validation. A) Active site position of four proteins analyzed using SCFBio webserver. B) Blind docking result, all inhibitor bound to proteins' active site

Molecular Docking Result

The docking results between CETP and the compounds revealed that two compounds exhibited the lowest binding affinity values and closely approached the inhibitors used as positive controls: rubranine and (-)-4-hydroxypanduratin A (Table 3). These two compounds formed bonds at the same residues as the inhibitor, namely Ile15, Val198, Phe441, and Phe461 (Fig. 2 and Table 4). The combination of their low binding affinity values and their binding positions identical to those of the inhibitor suggests that rubranine and (-)-4-hydroxypanduratin A have a high potential to act as CETP inhibitors. CETP plays a critical role in LDL formation by facilitating the transfer of cholesterol esters and triglycerides between HDL and LDL and VLDL, leading to the conversion of HDL into LDL or VLDL[5]. This CETP activity results in reduced HDL levels and elevated LDL levels, thereby increasing the risk of atherosclerosis[33]. Consequently, one approach to mitigating atherosclerosis is to inhibit the activity of the CETP protein[5].

Table 3. Binding affinity

Compound	CTEP	ACAT1	OSC	sPLA2
Inhibitor	-11.3	-9.3	-6.1	-8.2
(E)-geraniol	-5.8	-6.7	-4.8	-5.6
Dihydrochrysin	-7.8	-9.7	-7.5	-7.8
Sakuranetin	-7.6	-9.2	-7.1	-7.8
Isopimaric acid	-8.1	-9.6	-7	-7.3
Cardamomin	-7.2	-8.5	-6.7	-7.3
Flavokawin A	-6.8	-8	-6.1	-7.3
Boesenbergin A	-9	-10.1	-7.3	-8
Rubranine	-9.4*	-10.7*	-8.8*	-9.8*
Panduratin A	-9.3	-9.7	-8	-7.4
Alpinetin	-7.9	-9.6	-7.2	-7.9
5,7-Dihydroxy-8-C-geranylflavanone	-9.3	-10.7*	-8.5*	-8.7*
7,4'-Dihydroxy-5-methoxyflavanone	-7.2	-8.9	-7	-7.9
(-)-4-Hydroxypanduratin A	-9.4*	-9.6	-7.8	-7.7
Isopanduratin A	-9.1	-9.7	-7.5	-7.7
2,4-Dihydroxy-6-phenethyl-benzoic acid methyl ester	-7.1	-8.7	-6.6	-7.5
5,6-Dehydrokawain	-7.2	-8.4	-6.1	-7
7-Methoxy-5-hydroxy-8-geranylflavanone	-9.2	-9.6	-7.1	-8.6
(+)-Zeylenol	-8.6	-9.5	-7.6	-7.8
Crotepoxide	-7.3	-8.9	-6.8	-7.5

*: Indicate the lowest binding affinity values

The docking results for ACAT1-compound interactions revealed that Rubranine and 5,7-Dihydroxy-8-C-geranylflavanone exhibited the lowest binding affinity values. Rubranine formed three bonds at the same residues as the inhibitor, namely Phe381, Phe479, and Leu507 (Fig. 2E, F, and Table 4). On the other hand, 5,7-Dihydroxy-8-C-geranylflavanone formed the same four hydrogen bonds as the inhibitor at Leu377, Phe381, Trp420, and Phe479 (Fig. 2E, G, and Table 4). ACAT1 functions by transferring fatty acid groups from acyl-coenzyme A (Acyl-CoA) to the 3β-hydroxyl part of cholesterol, leading to the formation of cholesterol esters. These cholesterol esters then aggregate to create cytoplasmic lipid droplets within the[34]. Inhibiting ACAT1 activity has been shown to prevent the transformation of macrophages into foam cells[4]. Previous studies have suggested that inhibiting ACAT1 can be an effective strategy to prevent atherosclerosis by impeding foam cell formation[35].

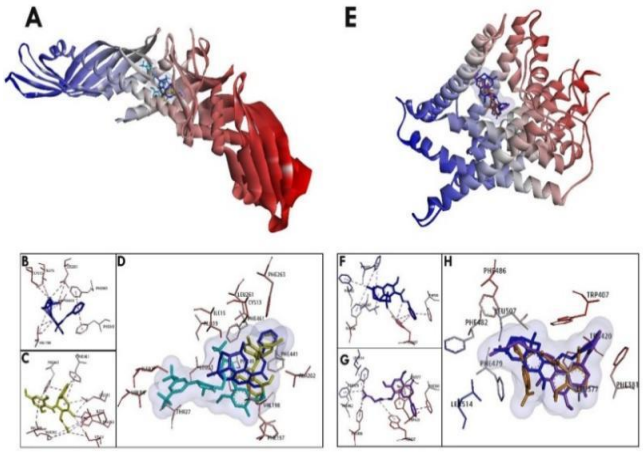

Figure 3. Protein-compounds interaction. The binding site of CTEP-compounds interaction (A). Details of interactions between CTEP-rubranine (blue) and CTEP-(-)-4-hydroxypanduratin A (yellow) (B and C). Comparison of interaction between compounds and inhibitors (anacetrapib) (Cyan) (D). Interaction between ACAT1 and compounds (E). Detail Interaction of ACAT1-rubranine (blue) and ACAT1- 5,7-dihydroxy-8-C-geranylflavanone (purple) (F and G). Comparison of the interaction between the compounds and the inhibitor (ART-101) (brown) in ACAT1 (H).

The results of the OSC-compound docking indicated that rubranine and 5,7-Dihydroxy-8-C-geranylflavanone had the lowest binding affinity values. Rubranine formed a bond at the same amino acid site as the inhibitor, namely Glu594 (Fig. 3A, B, and Table 4). On the other hand, 5,7-Dihydroxy-8-C-geranylflavanone bound to the same amino acids as the inhibitor, specifically at Glu398 and Lys46 (Figure 3A, C, and Table 4).

Based on these results, it can be concluded that rubranine and 5,7-dihydroxy-8-C-geranylflavanone have the potential to act as inhibitors of OSC. OSC plays a critical role in cholesterol synthesis, particularly in catalyzing the cyclization of 2,3-monoepoxysqualene to lanosterol and 2,3,22,23-diepoxysqualene to 24(S), 25-epoxylanosterol[7]. Inhibiting the activity of this protein has the potential to lower LDL levels in the plasma and prevent the accumulation of cholesterol in macrophages[36]. Previous studies have suggested that OSC inhibition could reduce cholesterol biosynthesis and potentially prevent atherosclerosis[37].

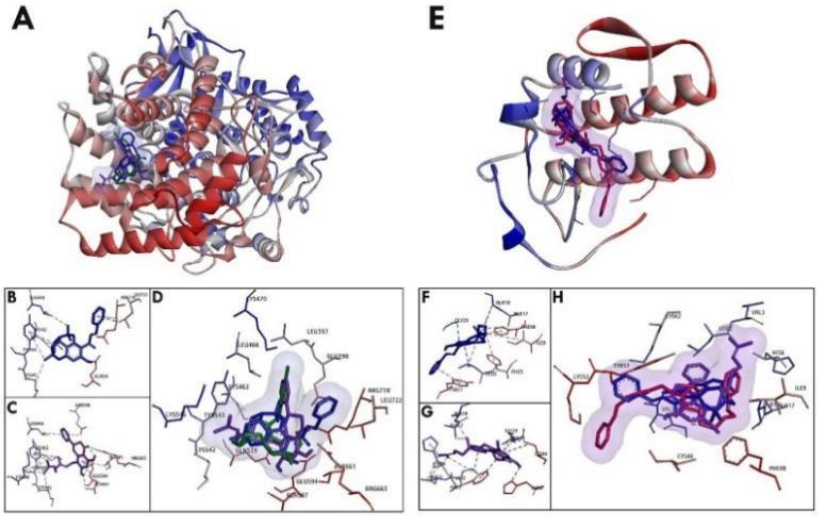

Figure 4. Protein-compounds interaction. The binding site of OSC-compounds interaction (A). Details of interactions between OSC-rubranine (blue) and OSC-(-)-4-hydroxypanduratin A (yellow) (B & C). Comparison of interaction between compounds and inhibitors (Ro 48-8071) (green) (D). Interaction between sPLA2 and compounds (E). Detailed interaction of sPLA2-rubranine (blue) and sPLA2- 5,7-dihydroxy-8-C-geranylflavanone (purple) (F & G). Comparison of interaction between the compound and the inhibitor (KH064) (red) in sPLA2 (H).

The interaction between sPLA2 and the compounds revealed that rubranine formed one hydrogen bond and six hydrophobic interactions with sPLA2 (Fig. 3E, F, and Table 4). On the other hand, 5,7-Dihydroxy-8-C-geranylflavanone formed one hydrogen bond and seven hydrophobic interactions. Rubranine bound to the same residues as the inhibitor, specifically at Gly29, Leu2, Ala17, and Ala18 (Fig. 3E, F, and Table 4). sPLA2 functions by hydrolyzing sn-2 ester bonds in glycerol phospholipids found in lipoproteins and cell membranes, resulting in the production of non-esterified fatty acids and lysophospholipids[38]. Both of these products can trigger inflammation leading to the development of atherosclerotic plaque[8]. Increased sPLA2 activity could induce the risk of atherosclerosis[39]. Previous studies suggested that inhibition of sPLA2 activity could prevent atherosclerosis[40]. In brief, medicinal plants compound had an essential role for therapeutic development[41]. Moreover, plants serve as rich sources of drug compounds in traditional medicine[42].

Table 4. Protein-ligand interaction in detail.

Protein	Ligand	Binding Affinity (kcal/mol)	Position of Chemical Interaction	
			Hydrogen bond	Hydrophobic interaction
CETP	Inhibitor (Anacetrapib)	-11.3	Thr27	Ile15, Ala19, Val74, Val84, Phe197, Val198, Phe441, Phe461, Phe463
	Rubranine	-9.4	-	Cys13, Ile15, Leu23, Val198, Leu261 Phe441, Phe461
	(-)-4-Hydroxypanduratin A	-9.4	-	Cys13, Ile15, Val198, Ala202, Leu261, Phe263, Phe441, Phe461
ACAT1	Inhibitor (ART-101)	-9.3	Trp420	Leu377, Phe381, Trp408, Phe479, Leu507
	Rubranine	-10.7	-	Phe381, Trp407, Phe479, Phe482, Phe486, Leu507
	5,7-Dihydroxy-8-C-geranylflavanone	-10.7	-	Leu377, Phe381, Trp407, Trp420, Phe479, Phe482, Phe486, Leu514
OSC	Inhibitor (Ro 48-8071)	-6.9	Ala661	Leu397, Glu398, Lys462, Lys470
	Rubranine	-8.8	Glu594	Lys462, Leu466, Lys542, Tyr543, Arg718, Leu722
	5,7-Dihydroxy-8-C-geranylflavanone	-8.5	Glu594, Arg663	Glu398, Lys462, Leu466, Lys542, Tyr543, Lys546, Ala597
sPLA2	Inhibitor (KH064)	-8.2	Gly29, Val30	Leu2, Ala17, Ala18, Gly31, Asp48, Lys52, Lys62
	Rubranine	-9.8	Gly29	Leu2, Phe5, Ile9, Ala17, Ala18, Tyr51, Phe98
	5,7-Dihydroxy-8-C-geranylflavanone	-8.7	Gly29	Leu2, Val3, Phe5, His6, Ala17, Ala18, Cys44

_: the same amino acids where inhibitors and compounds interact with protein

CONCLUSION

Flavonoids and essential oils were the predominant compounds found in fingerroot. All of the compounds present in fingerroot exhibit characteristics that make them suitable candidates for drug development. Seven compounds are predicted to possess anti-atherosclerosis activity, namely dihydrochrysin, sakuranetin, isopimaric acid, 2S-pinocembrin, 5,7-dihydroxy-8-C-geranylflavanone, 7,4'-dihydroxy-5-methoxyflavanone, 5,7-dihydroxy-8, and 7-methoxy-5-hydroxy-8-geranylflavanone. Several of these compounds bind to the active sites of atherosclerosis-related proteins (CETP, ACAT1, OSC, and sPLA2) with lower binding affinity values than the inhibitors. Based on this study, it can be concluded that fingerroot is predicted to have high potential as an anti-atherosclerosis agent by inhibiting the activity of the four atherosclerosis-related proteins. However, further research using in vitro and in vivo approaches is essential to confirm the exact anti-atherosclerosis potency of fingerroot.

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استكشاف المركبات النشطة بيولوجيًا المحتملة من Fingerroot (Boesenbergia rotunda L.) كمثبط للبروتينات المرتبطة بتصلب الشرايين (CETP،ACAT1، OSC ، sPLA2:) دراسة في السيليكون

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ملخص

يُعرف Boesenbergia rotunda L. (fingerroot) بأنه أحد النباتات الطبية الإندونيسية ذات الفاعلية الكبيرة في علاج الأمراض المختلفة، بما في ذلك تصلب الشرايين. تهدف هذه الدراسة إلى تحليل الفعالية المضادة لتصلب الشرايين للمركبات النشطة بيولوجيًا في جذور الأصابع من خلال تثبيط أربعة بروتينات مرتبطة بتصلب الشرايين (CETP و ACAT1 و OSC و sPLA2). المركبات النشطة بيولوجيًا من B. rotunda المسترجعة من قاعدة بيانات KnapSack، تم التنبؤ بخاصية تشابه الدواء باستخدام خادم الويب SwissADME، وتوقع النشاط الحيوي للمركب باستخدام خادم PASSOnline. تم إجراء تنبؤ الموقع النشط والتحقق من صحة البروتينات باستخدام خادم الويب SCFBio و Autodock Vina. تم إجراء الالتحام المحدد بين مركبات الإصبع والبروتينات بواسطة AutoDock Vina. Fingerroot يحتوي على 20 مركبًا حيويًا مع خصائص دوائية قوية. علاوة على ذلك، dihydrochrysin، sakuranetin، isopimaric acid، S-pinocembrin2، 5،7-dihydroxy-8-C-geranylflavanone، 4'-dihydroxy-5-methoxyflavanone7،4، dan 5-7-dihydroxy-8، و 7-methoxy-hydroxy-8-geranylflavanone-5 أنشطة مضادة لتصلب الشرايين. كان للروبرانين و (−) – 4–هيدروكسي باندوراتين A أقل درجة تقارب ملزمة مع CETP. اثنان من المركبات ذات أقل تقارب ارتباط في التفاعل مع ACAT1 و OSC و sPLA2 هما الروبرانين و 5،7–ثنائي هيدروكسي–8–سي–جيرانيل فلافانون. يمكن الاستنتاج أن جذر الإصبع لديه إمكانات عالية كعامل مضاد لتصلب الشرايين من خلال تثبيط 4 بروتينات مرتبطة بتصلب الشرايين على أساس نهج السيليكو.

الكلمات الدالة: ACAT1، تصلب الشرايين، CETP، الالتحام الجزيئي، OSC، sPLA2.

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Assessing the Influence of the COVID-19 Pandemic on the Purchasing Intention of Vitamins in Kuwait using the Theory of Planned Behavior

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ABSTRACT

Using the Theory of Planned Behavior model developed by Ajzen in 1985, the authors assessed the influence of attitude (ATT) toward vitamins, health awareness (HA), perceived behavioral control (PBC), subjective norms (SN), and knowledge of COVID-19 (KN) on the purchasing intention (PI) of vitamins in Kuwait during the COVID-19 pandemic. A total of 587 adults living in Kuwait completed the online survey, which was available in both Arabic and English. The measured variables included health awareness, attitude, knowledge about COVID-19, purchasing intention, perceived behavioral control, and subjective norms. The findings indicate that HA has a significant impact on ATT. Furthermore, the results revealed that HA significantly influences ATT, ATT has a significant influence on PI, KN has a significant influence on ATT, KN has a significant influence on PI, PBC positively influences PI, SN has a significant influence over PI, and SN positively influences PI.

Keywords: COVID-19, Kuwait, Vitamins, Theory of Planned Behavior.

INTRODUCTION

The coronavirus disease of 2019 (COVID-19) is a highly contagious respiratory disease caused by a novel coronavirus that was initially discovered in Wuhan, China, in December 2019. Some common symptoms of the disease include fever, dry cough, tiredness, myalgia, and dyspnea. Approximately 18.5% of Chinese patients progressed to the severe stage, where they developed acute respiratory distress syndrome, septic shock, difficult-to-treat metabolic acidosis, and bleeding and coagulation abnormalities [(1)]. It has been established that the pandemic's impacts extend beyond the psychological effects and high mortality rates of affected individuals. Mental health and lifestyle have also been affected [(2)].

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Indeed, the fatality rate of COVID-19 was 2.3% in China, which is much lower than those of Severe Acute Respiratory Syndrome (SARS) (9.5%), Middle East Respiratory Syndrome (34.4%), and Avian influenza A (39.0%). The COVID-19 epidemic spread very rapidly. By February 15, 2020, the COVID-19 virus had reached 26 countries in total, resulting in 51,857 laboratory-confirmed infections and 1,669 deaths, with nearly all infections and deaths occurring in China. Consequently, the World Health Organization (WHO) declared COVID-19 a public health emergency of international concern and called for collaborative efforts from all countries to prevent the rapid spread of COVID-19 [(3)]. The World Health Organization (WHO) classified COVID-19 as a pandemic on March 11, 2020 [(4)].

Kuwait recorded its first five confirmed COVID-19 cases imported from Iran on February 24, 2020. Kuwait has reported approximately 650,000 confirmed cases and approximately 2,500 deaths (source: Kuwait COVID -

Coronavirus Statistics - Worldometer) [5].

In research, behavioral theories are considered influential frameworks used to assess health-related behaviors. Human behavior has always been an intriguing question for researchers, leading many to explore factors influencing behavior and propose theories to explain it.

One of these theories is the Theory of Reasoned Action (TRA), developed by Fishbein & Ajzen in 1975. It is a social cognitive model that focuses on behavior intentions and behavior itself. This theory assumes that the behavior in question is under volitional control, meaning people believe they can execute the behavior whenever they decide to do so [6]. TRA takes into consideration individual motivational factors as determinants of the likelihood of carrying out specific behaviors. Initially, TRA was developed to investigate the connections between attitudes, intentions, and behaviors [7]. According to TRA, the attitude towards a behavior is considered a better predictor of the behavior than the attitude towards an object [8]. Furthermore, the Theory of Reasoned Action posits that behavioral intention is the most significant predictor of behavior. Both the attitude towards performing the behavior and the subjective norm associated with the behavior are factors that determine behavioral intentions [9]. The TRA theory is built on the following components:

1. Attitudes are defined as the positive or negative feelings resulting from the achievement of an objective. An attitude is influenced by behavioral beliefs, which are an individual's beliefs about the outcomes or attributes of performing a behavior in relation to evaluated outcomes and beliefs. For example, an individual with a strong belief and a positive valuation of the outcome will possess a positive attitude towards the behavior.

2. Subjective norms refer to normative beliefs held by individuals regarding their ability to achieve specific goals, influenced by the perceptions of their significant others, such as family members or spouses. Normative beliefs are weighted by the motivation to comply with these referents [9].

The Theory of Reasoned Action (TRA) highlights the idea that behaviors are under the total control of individuals who choose whether or not to execute them. However, this theory has limitations, as some behaviors are not under an individual's control [10]. The Theory of Planned Behavior (PB) is an extension of the TRA theory, suggesting that not all behaviors are entirely within an individual's control. Consequently, this extension of the TRA theory takes into consideration the level of control an individual has over their behaviors [11]. Additionally, the PB theory introduces perceived behavioral control as a fourth determinant of behavioral intentions. Perceived behavioral control relates to an individual's perception of the degree of control they have over performing a behavior. It is assessed by items that gauge an individual's confidence in engaging in the behavior and whether the execution of a behavior depends on the individual. Furthermore, the PB theory suggests that perceived behavioral control could have a direct influence on behavior [12]. The PB theory has been applied in various academic and research disciplines, including psychology, marketing, public relations, healthcare, sport management, and sustainability.

People who prioritize their health and well-being are often referred to as "health-conscious" individuals. These individuals proactively establish healthy routines and maintain awareness of their physical and mental health. They are driven to enhance and sustain their overall well-being and quality of life, with the additional goal of preventing illness [13,14].

Health-consciousness implies possessing a positive attitude toward healthy behaviors [15] and endorsing healthy choices [16]. Typically, individuals with high health-consciousness are more likely to purchase dietary supplements [17] and have a greater inclination to engage in healthy behaviors to preserve their health [13]. Attitude, encompassing an individual's range of emotions toward someone or something, has been demonstrated to influence

purchasing behavior. Research suggests that the stronger an individual's attitude toward dietary supplements, the more likely they are to intend to purchase these supplements, particularly among younger generations [18]. Moreover, the study indicates that individuals with health concerns are inclined to take preventive measures, such as regular exercise and dietary supplement consumption, more frequently than those without such concerns [18]. Dietary supplements are consumed by approximately 50% of adolescents in developed countries, with notably high usage rates in the United States of America (USA) and the United Kingdom (UK) [19] Approximately 54% of American adults use dietary supplements, including multivitamins and minerals [20]. Several studies have consistently identified attitude as one of the primary factors influencing behavior [21]. Furthermore, it has been suggested that individuals who develop a more positive attitude toward a behavior are more likely to engage in it [22]. Additionally, Basha and colleagues have established a positive relationship between attitude and the intention to purchase dietary supplements, with attitude influencing purchasing intention [23]. Kim and Chung (2011) and Newsom et al. (2005) found that health-conscious consumers exhibit a heightened awareness of their health and express genuine concern for it. Consequently, they actively engage in health-promoting behaviors and consistently strive to maintain and enhance their overall health and quality of life [24,25]. Gould (1988) identified a positive association between health consciousness and dietary patterns, particularly in terms of vitamin consumption [13].

According to a study that assessed individuals' dedication to maintaining their health behaviors, health-conscious consumers and athletes are increasingly turning to dietary supplements to enhance athletic performance and improve their overall health. This trend is driven by the growing demand for dietary supplements [26]. Additionally, it has been observed that individuals engaged in preventive behaviors, such as mask-wearing and practicing social distancing, during the COVID-19 pandemic. Social risk perception is positively linked to attitudes, perceived norms, and self-efficacy for these behaviors. However, personal risk perception is negatively associated with attitudes toward mask-wearing, as well as perceived norms and self-efficacy for both preventive behaviors [27].

Therefore, this article aims to investigate how the COVID-19 pandemic has influenced knowledge, attitudes, and practices, particularly concerning vitamin consumption. Furthermore, this article seeks to explore whether the Theory of Planned Behavior is applicable in the context of vitamin purchases during the COVID-19 pandemic.

Research aim:

Given the absence of related published research in Kuwait, the authors have undertaken to examine the impact of attitude (ATT) toward vitamins, health awareness (HA), perceived behavioral control (PBC), subjective norms (SN), and knowledge of COVID-19 (KN) on the intention to purchase vitamins (PI) in Kuwait during the COVID-19 pandemic.

1.MATERIALS AND METHODS

To analyze our extended theoretical model, we employed structural equation modeling (SEM), a well-established method widely utilized in various fields of research [28]. SEM is a technique that elucidates the interrelationships among multiple variables [29]. It encompasses various multivariate analysis techniques that enable the examination of systematic relationships between a set of predictors and dependent variables [30]. SEM can be applied through either the covariance-based approach (CB-SEM) or the variance-based approach (PLS-SEM) [31]. PLS-SEM is adept at handling modeling challenges that frequently arise, such as unconventional data characteristics and highly complex models [31]. In this study, we utilized PLS-SEM, leveraging SmartPLS 3 due to the small sample size [31]. Anderson and Gerbing, 1988

recommended using two-stage analytical procedures, by firstly evaluating the measurement model and then assessing the structural model, to test the hypothesized relationship [32]. To determine the significance of the path coefficients, we employed a bootstrapping method [31].

1.1 Participants

The study was based on data collected online via a survey distributed from November 30, 2021, to January 1, 2022. The target population included residents of Kuwait who were both Arabic and English speakers. The study sample comprised 587 Kuwaiti adult residents currently residing in Kuwait (n = 587). Researchers employed a non-probability sampling method, specifically a convenience sampling method, to collect data from these 587 respondents. The questionnaire was distributed through online channels such as Facebook, WhatsApp, and Instagram to facilitate participation. This approach allowed researchers to efficiently and effectively gather a substantial amount of primary source data for the study.

1.2 Data collection techniques

The data were collected online using a structured questionnaire developed after a comprehensive review of the relevant literature. The questionnaire comprised items related to the study's highlighted variables, including health awareness, knowledge of COVID-19, attitude toward vitamins, subjective norms, perceived behavioral control, and the intention to purchase vitamins. Multiple-item scales were designed following the recommendations of Ajzen and Fishbein to measure these variables. A five-point Likert scale was employed to gauge the extent to which participants agreed or disagreed with a set of statements assessing the variables in the study (1= strongly disagree to 5= strongly agree). Statements from prior research were adapted to measure the constructs in the current study. The accuracy, clarity, content validity, relevance, and conciseness of the questionnaire items were assessed by three academics and researchers in the field of pharmacy in Jordan (Associate Prof. Yazun Jarrar, Prof. Amal Akour, Ph.D., and Ph. Ruba Balasmeh). Recommended revisions were deliberated and incorporated before finalizing the questionnaire. Table 1 presents the survey statements included in the questionnaire. A pilot study involving 50 participants was conducted to assess the questionnaire's validity and reliability. Consequently, the number of statements was reduced, and some statements were rephrased to enhance understanding among the target population. Furthermore, Cronbach's alpha indicated good to excellent reliability, ranging from 0.827 to 1 (Table 1).

The questionnaire was distributed in two versions: one in Arabic and one in English. A professional certified translator fluent in English, German, and Arabic (Deena Moghrabi) translated the questionnaire into Arabic. After minor revisions and adjustments, the two versions were aligned for consistency. The English version had 164 respondents, while the Arabic version had 385 respondents. Given that most participants were more proficient in Arabic than in English, having both versions of the questionnaire were deemed necessary.

Table 1. Questionnaire statements

Construct	Cronbach alpha coefficient	Statements	Source
Health awareness	0.827	I reflect on my health a lot I am very self-conscious about my health I am alerted to changes in my health I take responsibility for the state of my health	(Michaelidou & Hassan, 2008) (Gould, 1988)
Knowledge of Covid-19	1.000	Boosting immunity by consuming vitamins helps in the prevention from Covid-19	(Chi, 2021) (Pop et al., 2020)
Attitude	0.850	I think money spent on vitamins is worthwhile It is important to take vitamins It is useful to take vitamins	(Ajzen, 1991) (Pop et al., 2020)
Purchasing intention	0.928	I want to purchase vitamins within the next two weeks I intend to purchase vitamins within the next two weeks	(Ajzen, 1991)
Perceived behavioral control	1.000	If I wanted, it would be easy for me to buy vitamins	
Subjective norm	0.898	Most people who are important to me think I should purchase vitamins People whose opinions I value would prefer me to purchase vitamins	

1.3 Model structure

The initial structural model comprises one dependent variable (the intention to purchase vitamins) and five independent variables (attitude toward vitamins, health awareness, perceived behavioral control, subjective norm, and knowledge of COVID-19), all of which are considered latent constructs.

This study employed a range of observed variables to measure these latent variables. Questionnaire statements previously used in related research and studies were adapted to the current research context.

The questionnaire is divided into two sections. The first section includes several questions concerning respondents' demographic information, while the second section comprises indicator items for each construct. Health awareness was assessed using four questions, knowledge of COVID-19 was assessed using one question, attitude was assessed using three questions, purchase intention was assessed using three questions, perceived behavioral control was assessed using one question, and subjective norms were assessed using two questions. All questionnaire statements are detailed in Table 1.

2. RESULTS

2.1 Profile of respondents

Table 1 provides an overview of the sample used in this study. Of the respondents, 63.2% were female, 35.3% were male, and 1.5% chose not to disclose their gender. Regarding educational attainment, 50% of respondents were pursuing a bachelor's degree, 38.2% were pursuing a

master's degree, and 11.8% were pursuing a Ph.D. degree. It's worth noting that this research does not investigate the potential mediating influence of socioeconomic and demographic factors on the research variables; therefore, additional demographic data were not collected.

Table 2. Demographic characteristics of respondents

Sample characteristics	Respondents (n=204)	Frequency (%)
Gender		
Female	434	74.06 %
Male	152	25.93 %
Educational level		
Bachelor	422	72.01 %
Master	117	19.96 %
PhD	47	8.02 %

2.2 Assessment of the measurement model:

Table 2. Loadings, Reliability, and Validity

	Loadings	Cronbach's Alpha	Composite reliability	AVE
ATT1	0.835	0.850	0.909	0.770
ATT2	0.915			
ATT3	0.879			
HA1	0.787	0.827	0.885	0.659
HA2	0.839			
HA3	0.860			
HA4	0.756			
PI1	0.965	0.928	0.965	0.933
PI2	0.967			
KN1	1.000	1.000	1.000	1.000
PBC1	1.000	1.000	1.000	1.000
SN1	0.948	0.898	0.951	0.907
SN2	0.957			

Remark: ATT1= attitude question 1, ATT2= attitude question 2, ATT3= attitude question 3, HA1= health awareness question 1, HA2= health awareness 2, HA3= health awareness 3, HA4= health awareness question 4, PI1= Purchase intention question 1, PI2= purchase intention question 2, KN1= Knowledge question 1, PBC1= perceived behavioral control question 1, SN1= subjective norms question 1 , SN2= subjective norms question 2.

As part of evaluating the measurement model, no items were removed from the analysis because their factor loadings were higher than 0.600 (<0.600) (Gefen and Straub, 2005) [33]. To test the reliability of the constructs, the study used Cronbach's alpha and composite reliability (CR). All the CRs were higher than the recommended value of 0.700 [31]. Cronbach's alpha for each construct exceeded the 0.700 threshold. Convergent validity was acceptable because the

average variance extracted (AVE) was over 0.500. The results for reliability and validity, in addition to the factor loadings for the items, are presented in Table 2. Discriminant validity was assessed by the Fornell-Larcker criterion. The table shows that the square root of AVE for each construct was greater than the inter-construct correlations (Table 3). Discriminant validity was also evaluated by the Heterotrait-Monotrait ratio of correlations [34], with values below the threshold of 0.90. Consequently, discriminant validity is established (see Table 4).

Table 3. Fornell-Larker Criterion

	ATT	HA	KN	PBC	PI	SN
ATT	**0.877***					
HA	0.247	**0.812***				
KN	0.609	0.218	**1.000***			
PBC	0.283	0.241	0.290	**1.000***		
PI	0.580	0.241	0.471	0.310	**0.966***	
SN	0.460	0.169	0.401	0.288	0.522	**0.953***

*Remark: AT1= attitude question, HA= health awarenessKN= Knowledge, PBC= perceived behavioral control, SN= subjective norms, *= Square-root of AVE.*

Table 4. HTMT ratio

	ATT	HA	KN	PBC	PI	SN
ATT						
HA	0.293					
KN	0.661	0.241				
PBC	0.307	0.265	0.290			
PI	0.652	0.274	0.490	0.322		
SN	0.525	0.197	0.422	0.304	0.571	

Remark: ATT= attitude, HA= health awareness, KN= Knowledge, PBC= perceived behavioral control, SN= subjective norms.

2.3 Assessment of the structural model

The structural model indicates the hypothesized paths in the research framework. The structural model is assessed based on R^2, Q^2, and the significance of paths. A 40.3% change in purchasing intention can be attributed to attitude towards vitamins, knowledge of Covid-19, subjective norms, and perceived behavioral control. A 29.1% change in the attitude towards vitamins is attributed to health awareness and knowledge of Covid-19. Both R2 values are greater than 0.1 [35]. Consequently, the predictive capability is established. Q2 for attitude towards vitamins and the purchasing intention of vitamins is higher than 0, which means that the model has predictive relevance. The value of SRMR was 0.048, which is below the required value of 0.20, indicating an acceptable model fit [31].

Further assessment of the goodness of fit, and hypotheses were tested to establish the significance of the relationships. H1 evaluates whether HA has a significant impact on ATT. The results revealed that HA does have a significant impact on ATT ($\beta = .120$, $t = 3.503$, $p > .01$). Hence, H1 was accepted. H2 assesses whether ATT has a significant influence on PI. The results showed that ATT has a significant impact on PI ($\beta = .352$, $t = 8.883$, $p < .01$). Consequently, H2 was accepted. H3 studies whether KN

has a significant influence on ATT. The results showed that KN has a significant influence on ATT (β = .583, t = 17.875, p < .01). Therefore, H3 was accepted. H4 studies whether KN has a significant influence on the PI. The results demonstrated that KN has a positive influence on PI (β = .114, t = 3.099, p < .01). Therefore, H4 was accepted. H5 assesses whether PBC has a significant impact on PI. The results demonstrated PBC has a positive influence on PI (β = .095, t = 2.676, p < .01). Hence, H5 was accepted. H6 evaluates whether SN has a significant influence over PI. The results demonstrated that SN positively influences PI (β = .288, t = 6.761, p < .01). Consequently, H6 was accepted.

The 5000 resamples of the study generated 95% confidence intervals as shown in Table 4. A confidence interval different from zero indicates a significant relationship. Hypothesis testing results are summarized in Table 5.

Table 5. Hypothesis testing results

	B	STDEV	T Statistics	P Values	2.5%	97.5%
ATT -> PI	0.352	0.040	8.883	0.000	0.276	0.426
HA -> ATT	0.120	0.034	3.503	0.001	0.040	0.179
KN -> ATT	0.583	0.033	17.875	0.000	0.520	0.645
KN-> PI	0.114	0.037	3.099	0.002	0.044	0.186
PBC-> PI	0.095	0.035	2.676	0.008	0.031	0.163
SN-> PI	0.288	0.043	6.761	0.000	0.212	0.377
	R^2	Q^2				
ATT	0.385	0.291				
PI	0.438	0.403				

Remark: ATT= attitude, PI= purchase intention, HA= health awareness, KN= Knowledge, PBC= perceived behavioral control, SN= subjective norms.

2.4 Mediation analysis

Mediation analysis was conducted to assess the mediating role of attitude towards vitamins. The results (see Table 6) indicated a significant (p < 0.01) mediating role of ATT (β = 0.205, t = 7.449, p = 0.002) and (β = 0.042, t = 3.115, p = 0.000). Hence, attitude towards vitamins successfully mediated the relationship between the knowledge of Covid-19 and purchasing intention and mediated the relationship between health awareness and the purchasing intention of vitamins. Thus, H7 and H8 were confirmed.

Table 6. Mediation results

	Total Effect	T	Sig	Direct effect	Sig		Effect	T statistics	P value
KN->PI	0.319	3.115	0.000	0.230	0.000	KN->ATT->PI	0.205	7.449	0.000
HA->PI	0.042	8.799	0.000	0.042	0.000	HA->ATT->PI	0.042	3.115	0.002

Remarks: KN= knowledge, PI= purchase intention, HA= health awareness

3. DISCUSSION

In accordance with the assumptions of the Theory of Planned Behavior (TPB), the findings of the present study reveal significant associations between the attitude (ATT) toward vitamins, perceived behavioral control (PBC), and subjective norms (SN) on the purchasing intention (PI) of vitamins among Kuwaiti participants during the COVID-19 pandemic as

precautionary measures aimed at preventing infection from the virus in Kuwait. These findings align with the Theory of Planned Behavior (TPB). They are consistent with a cross-sectional study conducted by Liu et al. in 2021 in Wuhan, China, which demonstrated that attitudes, subjective norms, and perceived behavioral control positively influenced the intention to purchase dietary supplements [36].

Another study applied the TPB to the use of multivitamins in a population of Caucasian female college students and found that attitude and perceived behavioral control had a statistically significant impact on the intention to use multivitamins [37]. A master's thesis conducted at Eastern Michigan University in the United States confirmed that subjects' attitudes and perceived behavioral control were associated with the intention to use multivitamins [38].

The study findings are further consistent with Alami et al. (2019), as their results provided support for the effectiveness of TPB and its potential constructs in testing for the determinants of iron and vitamin D supplement intake among adolescents in Iran [39]. We found that knowledge, subjective norms, attitude, and PBC could be potential determinants to explain and predict female adolescents' intentions regarding vitamin D and iron consumption. PBC was the strongest construct of TPB at predicting people's intentions to use iron and vitamin.

Furthermore, the findings of the present study show that there are significant associations between health awareness (HA) and the knowledge of COVID-19 (KN) on the purchasing intention (PI) of vitamins among Kuwaiti participants during the COVID-19 pandemic as precautionary measures aimed at preventing infection from the virus. This demonstrates that Kuwaitis are well aware of the health guidance provided in the FAO report [40]. These findings are consistent with two other studies that demonstrated people's lifestyle behaviors changed in order to stay healthy during the influenza and cold season. They considered healthy nutrition, such as eating vitamin-rich foods and taking complex vitamin supplements [41,42] Furthermore, this intention of buying and consuming vitamins adopted by Kuwaiti participants during the COVID-19 pandemic reflects the extent to which people follow the news and adhere to the health guidance from the WHO on COVID-19. The WHO has declared several effective health precautions to prevent the COVID-19 pandemic, such as social and physical distancing, avoiding crowded public places, using cleaning wipes that contain at least 60% alcohol or chloride, wearing a cloth covering when outdoors, maintaining a distance of at least 6 feet from others, and washing hands with soap for at least 20 seconds, along with maintaining a healthy diet rich in vitamins and minerals[43].

4.CONCLUSION

The current study expanded upon the use of TPB by incorporating the knowledge of Covid-19 and health awareness into the Theory of Planned Behavior (TPB). We aimed to measure the influence of attitude (ATT) towards vitamins, health awareness (HA), perceived behavioral control (PBC), subjective norms (SN), and Covid-19 knowledge (KN) on vitamin purchasing intention (PI) in Kuwait during the COVID-19 period.

The findings revealed that health awareness (HA) significantly influences attitude (ATT), attitude (ATT) significantly influences purchase intention (PI), knowledge (KN) significantly influences attitude (ATT), knowledge (KN) significantly influences purchase intention (PI), perceived behavioral control (PBC) positively influences purchase intention (PI), subjective norms (SN) significantly influence purchase intention (PI), and subjective norms (SN) positively influence purchase intention (PI).

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تقييم تأثير جائحة COVID-19 على نية شراء الفيتامينات في الكويت باستخدام نظرية السلوك المخطط

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ملخص

باستخدام نظرية نموذج السلوك المخطط الذي طوره أجزين في عام 1985، قام المؤلفون بقياس تأثير السلوك تجاه الفيتامينات والوعي الصحي والتحكم السلوكي الملحوظ والمعايير الذاتية ومعرفة فايروس كورونا المستجد بخصوص نية شراء الفيتامينات في الكويت خلال فترة انتشار الفايروس. أكمل 587 شخصًا بالغًا يعيشون في الكويت الاستبيان الموزع عبر الإنترنت باللغتين العربية والإنجليزية. وتضمنت المتغيرات التي تم قياسها الوعي الصحي والسلوك والمعرفة بالفايروس ونية الشراء والتحكم السلوكي المتصور والمعايير الذاتية. وتشير النتائج إلى أن الوعي الصحي له تأثير كبير على السلوك. وكشفت النتائج أن الوعي الصحي له تأثير كبير فعلاً على السلوك، وأن السلوك له تأثير كبير على نية الشراء، وأن المعرفة لها تأثير كبير على السلوك، والمعرفة لها تأثير كبير على نية الشراء، والتحكم السلوكي المتصور له تأثير إيجابي على نية الشراء، والمعايير الذاتية لها تأثير كبير على نية الشراء، والمعايير الذاتية تؤثر إيجابياً على نية الشراء.

الكلمات الدالة: كوفيد -19، الكويت، فيتامينات، نظرية السلوك المخطط.

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Assessment of the Fungicidal and Nematicidal Potential of *Reichardia tingitana* (L.) Roth on Phytopathogenic Fungi and Plant Nematode

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ABSTRACT

The primary concern was the removal of numerous soil fumigants and nematicides due to their potential risks to human and environmental safety. Fungal pathogens can cause serious diseases in humans and animals. Among these, root-knot nematodes such as Meloidogyne incognita and Tylenchulus semipenetrans pose a significant threat, leading to substantial damage and yield reduction in various economically important plants. Therefore, this study aimed to assess the fungicidal and nematicidal activities of the ethanol extract (EE) and lupeol (L), the major isolates from the aerial parts of Reichardia tingitana L. Roth (Asteraceae), against Aspergillus flavus and plant-parasitic nematodes. Antifungal actions of EE (10-120 ppm) and L (23.4-281.2 µM) were evaluated through in vitro and in vivo growth assays, spore germination inhibition assays, and the efficacy of inhibiting pod and kernel infection. Nematicidal activity of EE and L was tested by preparing cultures containing egg masses of nematode species M. incognita from infected eggplants and T. semipenetrans from infected citrus roots, using concentrations of 2.5, 5, 10, 20, 40, 80, and 120 ppm. Results showed that R. tingitana (EE) and (L) exhibited nematostatic or nematicidal effects on nematode viability, egg hatch in vitro, and development and reproduction in vivo. Lupeol was particularly effective in inhibiting the colonization of A. flavus in peanuts. EE and L demonstrated high toxicity against nematodes in laboratory exposure and were effective in controlling nematode infestation in eggplant roots for 45 days. Improvement in plant growth parameters, including shoot and root length and weights, varied and was proportional to the doses of EE and L treatments. The antifungal and bio-nematicide effects of the ethanol extract from the aerial parts of R. tingitana were superior to those of lupeol, which could be attributed to the synergistic effect of phytochemicals in the ethanol extract. Both EE and L have potential applications as antifungal and bio-nematicide agents.

Keywords: Aflatoxin, Aspergillus flavus, false sow-thistle, lupeol, plant nematode, triterpenes.

List of Abbreviations: EE: Ethanol extract, CC:Column chromatography. L:Lupeol, LC50: the median lethal concentration (LC50) values,TLC: thin-layer chromatography,S1:System 1, TMS : Tetramethylsilane as an internal standard , PDA: potato dextrose agar medium, DC: Diameter of the colony in the control (mm), DT: Diameter of the colony in the treatment (mm), NC: Number of the fungal colony in control, NT: Number of the fungal colony in treatment, PF: Final Population, PI: nematode initial Population.

HIGHLIGHTS

- The study investigates the fungicidal and nematicidal activities of Reichardia tingitana L. Roth (Asteraceae) against phytopathogenic fungi and plant-parasitic nematodes.
- The ethanol extract of the aerial parts of R. tingitana (EE) and lupeol, a major isolate (L), exhibited nematostatic or nematicidal effects on nematode viability, egg hatch in vitro, and development and

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reproduction in in vivo applications.

- Lupeol effectively inhibits the colonization of A. flavus in peanuts.

1. INTRODUCTION

The genus Reichardia belongs to the dandelion family Asteraceae and is native to the Mediterranean and western Asia. Reichardia tingitana L. Roth, also known as false sow-thistle, has been reported to contain volatile oil, triterpenes, sterols, and sesquiterpenes. [1] This plant exhibits various beneficial properties, including antioxidative, anti-diabetic, anti-inflammatory, and antiviral activities. [2] Additionally, studies have investigated its insect deterrent and insecticidal properties, particularly regarding guaianolides. [3] Triterpenes were previously reported to exert an antifungal activity [4], and Lupeol was reported as inhibitors for both fungal growth and mycotoxin production of toxigenic *Fusarium* species. [5]

Peanut (Arachishypogaea) is a significant cash crop in tropical and subtropical countries. However, due to its pods being in contact with the soil, peanuts are vulnerable to fungal pathogens. One of the most common storage fungi that colonize peanuts is Aspergillus flavus. This fungus is capable of causing seed rots, mold growth on seeds, pre- and post-emergence damping-off, and reducing both seed viability and seedling growth in peanuts. [6] Mycotoxins are produced by Aspergilli and can contaminate not only food but also feedstuffs. It has been reported that at least 25% of the grain produced worldwide each year becomes contaminated with mycotoxins. Among these mycotoxins, aflatoxins, produced by A. flavus and A. parasiticus, are particularly notorious for their carcinogenic and immunosuppressive effects. Aflatoxins are toxic, low-molecular-weight metabolites that can harm plants, animals, and microorganisms. [7] In nature, at least 14 different aflatoxins are produced, with Aflatoxin B1 being the most toxic. Both A. flavus and A. parasiticus can produce Aflatoxin B1. [8,9] These fungi commonly infect various crops, including peanuts, tree nuts, and wheat. [10] Additionally, they pose a serious threat to the health of animals and humans, causing problems such as teratogenicity, immunotoxicity, and hepatotoxicity. [11] All animal species in addition to adult humans are resistant to the acute toxicity of aflatoxins also have a high tolerance for aflatoxin exposure and rarely yield acute aflatoxicosis. [12] However, children are particularly affected, and their exposure can lead to stunted growth and delayed development. [13]

Fungal pathogens have the potential to cause serious diseases in both humans and animals. [14] However, due to concerns regarding the toxicity of existing antifungal agents and the emergence of drug-resistant strains, there is growing interest in exploring alternative methods to control these pathogens, such as the use of plants. Many plants contain secondary compounds that have been found to have inhibitory effects on harmful bacterial and fungal pathogens that affect humans. [15,16]

Eggplant (Solanum melongena L.) is one of the most important and commonly cultivated vegetable crops worldwide. Belonging to the Solanaceae family, it is extensively grown in regions such as Asia, Egypt, and the Middle East. [17] Eggplants are a rich source of essential vitamins like C, K, B6, niacin, thiamin, as well as essential nutrients including magnesium, phosphorus, copper, dietary fiber, folic acid, potassium, and manganese. However, this crop is highly susceptible to pollution caused by root-knot nematodes, particularly those of the genus Meloidogyne, such as M. incognita. [18] While several nematicides are available for managing the root-knot nematode crisis affecting vegetables like eggplant, their use is expensive and poses environmental hazards. [19]

Traditionally, nematicides have been applied to control nematodes. However, there has been growing concern over the removal of numerous soil fumigants and nematicides due to their potential risks to human and environmental safety. [20] An alternative approach involves using plants as sources of compounds for sustainable management of plant-parasitic nematodes. This approach focuses on economically significant nematode species,

such as root-knot nematodes of the genus Meloidogyne, which have a wide range of hosts and pose threats to various annual and perennial crops. [21] In our study, we found that the roots of Pulsatilla Koreana exhibited strong nematicidal activity against Meloidogyne incognita after 48 hours, with an LC50 of 92.8µg/ml. This suggests that triterpenoid saponins from P. Koreana have the potential to be explored as natural nematicides for developing new agents to control root-knot nematodes. [22] Additionally, the citrus nematode T. semipenetrans is considered the most predominant and economically important pathogen causing significant damage to citrus trees in orchards and nurseries. [23] In the current study, we evaluated the effects of the ethanol extract (EE) and lupeol (L) isolated from R. tingitana on the colonization of Aspergillus flavus in peanuts. We also investigated their nematicidal effects on egg masses of nematode species Meloidogyne incognita, found in infected eggplants, and Tylenchulus semipenetrans, found in infected citrus roots.

2. MATERIAL & METHODS

2.1. Plant material

Aerial parts of *Reichardia tingitana* were obtained from Benghazi, Libya and identified by Dr. Reem Samir Hamdy, Professor of Plant Taxonomy, Botany Department, Faculty of Science, Cairo University, Giza, Egypt. At the Museum of the Pharmacognosy Department, Faculty of Pharmacy, Cairo University, https://goo.gl/maps/v6PsvJp6KJW52PkH8, the sample of the plant code 2015419 is deposited.

2.2. *Extraction*

The air-dried powdered aerial parts of R. tingitana (500g) were extracted by cold percolation with 95% ethanol (3L) until exhaustion. The ethanol extract was evaporated under reduced pressure, resulting in 120g of greenish-brown semi-solid residue. The dried residue (50g) was then suspended in distilled water and successively partitioned between *n*-hexane, chloroform, and *n*-butanol saturated with water, yielding 13g, 1g, and 7g, respectively.

2.3. Separation of the components of the *n*-hexane extractive

On a silica gel column, the n-hexane extract (10g) was chromatographed using *n*-hexane and *n*-hexane-ethyl acetate mixtures as the eluent. Twenty milliliters of each fraction were examined by TLC. Fractions that showed major spots were chromatographed on a silica gel column using n-hexane-ethyl acetate mixtures as the eluent. Lupeol was isolated as the major compound, identified by spectral data and comparison with previously reported data. [4] Both the ethanol extract of the aerial parts and the main isolate were subjected to tests to evaluate their antifungal and nematicidal effects.

2.4. Estimation of the antifungal effect

2.4.1. Isolation of fungal pathogen:

Peanut pods were cut into small pieces (2-5mm), and kernels were disinfected for three minutes using 2.5% sodium hypochlorite, rinsed three times in sterile distilled water, and then dried between layers of sterile filter paper (Whatman, No. 1). Ten groups of peanut pod pieces and kernels, each with five pieces of pods and kernels, were plated out on 15 ml of potato dextrose agar (PDA) medium (5 pieces of pod or kernel per plate) and then incubated at 25°C for 6 days. Subculturing was repeated several times to obtain pure cultures from mycelial tips, which were preserved on PDA slants until identification. The isolated A. flavus was identified based on colony and morphological characteristics. [24] The culture of the A. flavus strain was preserved on a PDA slant in the lab.

2.5. In vitro growth inhibitory assay

The stock solution of each R. tingitana sample (EE and L) was prepared by adding it to the PDA medium to achieve concentrations of 10-120 ppm. The medium was poured into Petri dishes and inoculated with equal discs (9mm in diameter) of the A. flavus pathogenic fungus. Plates containing mycelium discs without plant material were used as a negative control. All plates were incubated at 28 ± 2°C for 4 days. Fungal growth was measured as colony diameter, and the toxicity of plant materials against

A. flavus was expressed as the percentage of mycelia inhibition using the formula: Inhibition of Growth (%) = (DC – DT) / DC × 100, where DC represents the diameter of the colony in the control (mm) and DT represents the diameter of the colony in the treatment (mm), as defined by Ismail et al. [25].

2.6. Spore germination inhibition assay

Fungal spores were collected using sterile distilled water containing 0.1% Tween 80, from PDA plates after 7 days of growth at 28°C. Final concentrations of spores were adjusted to 1.45 × 106 spores/ml. for the assay; autoclaved PDA medium (20ml) was mixed with concentrations of (10 - 120 ppm) of samples of EE, L, and 1.45 × 106 spores and then poured into Petri plates (10cm diameter). After solidification, plates were incubated at 28 ± 2 °C for 4 days. The percent germination reduction of spores was determined by the next formula:

Inhibition of spore germination (%) = NC –NT/NC×100

Where, NC: Number of the fungal colony in control, NT: Number of the fungal colony in treatment.

In vivo efficacy inhibition of pod and kernel infection

Peanut pods and kernels (susceptible variety Giza 3) were surface sterilized by soaking them in a 0.1% aqueous solution of mercuric chloride for 3 minutes, rinsed with sterile distilled water, and then five pods and kernels each were placed separately in a sterile Petri dish (10 cm diameter) on filter paper. Six replicates with five pods and five kernels each were maintained. These peanut pods and kernels were treated with a requisite concentration of 120 ppm of EE and 281.2µM of L separately and inoculated with A. flavus by gently applying a conidial spore suspension (1×106 spores/ml) to the surfaces of the pods and kernels, followed by incubation at 25°C. To maintain high humidity, sterile distilled water (1-2 ml) was added every day during the first five days. After six days of incubation, the percentage incidence of A. flavus in the treated and untreated samples was determined by counting the number of kernels contaminated by A. flavus. The selection of concentrations was based on effectiveness, with a focus on choosing the lowest concentrations that could be used effectively, with their effects exceeding 50% on nematodes. This allowed them to be relied upon in establishing the toxicity threshold and calculating LC50.

2.7. Evaluation of the nematocidal activity

2.7.1. Nematode culture

Culture preparation of egg masses from each nematode species, root-knot nematode (M. incognita) from infected eggplant, and T. semipenetrans from infected citrus roots, was performed. Root tissues were placed in tap water for egg hatching. The egg suspension was poured onto cotton-wool paper and incubated at 28±2°C to obtain freshly hatched juveniles (J2). Juveniles were collected within 48 hours and used. [26]

2.7.2. Mortality test

A stock solution of each R. tingitana sample (EE) and (L) at concentrations of 2.5, 5, 10, 20, 40, 80, and 120 ppm was prepared by soaking the specified amount of each material (EE and L) in distilled water. One milliliter of nematode suspension containing 100 freshly hatched juveniles of Meloidogyne incognita and Tylenchulus semipenetrans was added to a fixed volume of the samples in Petri dishes (80mm). Additionally, 100 freshly hatched second-stage larvae of M. incognita were placed in 5ml of distilled water as a control. All dishes were incubated in an incubator at (25±2°C). After 24, 48, and 72 hours, the juveniles were counted for mortality and non-mortality under a stereoscope microscope. The death of nematodes was confirmed by keeping them in tap water for 24 hours. The percent mortality was calculated from an average of three replicates.

2.7.3. Greenhouse experiment

A greenhouse experiment was conducted to study the effect of R. tingitana on the nematode population of Meloidogyne incognita. One-month-old eggplant seedlings, Solanum melongena cv. White Balady, with uniform size, were transplanted singly into 15cm clay pots filled with sandy clay soil (2:1, v:v) at rates of 120, 80, and 40 ppm per pot. One week later, each pot was inoculated

with 2000 freshly hatched juveniles of Meloidogyne incognita. Each treatment was replicated three times.

All treatments were arranged in a completely randomized scheme under greenhouse conditions at a temperature of 35±2°C. Additionally, three control treatments were performed, including the bio-product (The tested Micronema (Bionematicide containing bacteria, supplied by the Agriculture Research Center, Giza, Egypt, from DOTRA Co, Haram, Giza, Egypt) applied at recommended rates as a soil drench by suspending the required quantity in 12ml. One week later, each pot was inoculated with 2000 freshly hatched juveniles of M. incognita. There were also untreated infected plants and untreated plants. Pots were watered periodically every two days, and the plants were harvested after 45 days from the time of inoculation.

2.7.4. Percentage reduction in nematode enumeration and plant growth parameters determination

The soil of each pot was processed for nematode extraction using the sieving and Baerman–pan technique (27). The count of second-stage juveniles (J2) within the soil of every pot was determined using a Hawksley counting slide and a stereoscopic microscope. Similarly, average records of eggs/egg masses were determined by washing four randomly selected egg masses per root system of every replicate in 1% sodium hypochlorite to release eggs from the egg matrix. The released eggs were then suspended in water and counted using a stereoscopic microscope. Galls and egg masses and their indices were evaluated, and collected juveniles were counted. The reduction percentage in gall formation, egg mass production, as well as female and juvenile numbers, were calculated using the following formula:

$$R\% = \frac{\text{Treatment - Control infected}}{\text{Control infected}} \times 100$$

The final population and nematode build-up were calculated for all treatments. The final population (PF) included the total number of juveniles in the soil, egg-masses, and females. The rate of build-up, denoted as (PF/PI), was determined by dividing the nematode final population (PF) by the nematode initial population (PI) (PI). [28] Plant growth responses, including shoot length, fresh and dry shoot weights, as well as root fresh weight and length, were measured and calculated for all treatments.

The data were subjected to analysis of variance (ANOVA), as outlined by Snedecor and Cochran. [29] Treatment means were compared using Duncan's Multiple Range Test at a 5% level of probability. [30] These analyses were conducted using SPSS Program version 16.

3. RESULTS

3.1. Isolation and identification of the major component of *n*-hexane extract

Lupeol was isolated as the major phytochemical in the hexane-soluble fraction of the ethanol extract from the aerial parts of R. tingitana. Its identity was confirmed based on spectral data; ^{1}HNMR and ^{13}CNMR (400MHz, and 100 MHz $CDCl_3$) which are presented in Table 1 and compared with previously reported data. [4] Lupeol was obtained as white needle crystals (50 mg) and crystallized from methanol with a melting point of 210-212°C. It gave a positive response in the Liebermann–Burchard test, indicating the presence of a triterpenoid skeleton. A violet color was observed with P-anisaldehyde / H_2SO_4 spray reagent. The IR spectrum exhibited absorption bands of hydroxyl group at 3415 cm^{-1} as well as olefinic bond absorption band at 1642, 880 cm^{-1} and 2945, 2869 cm^{-1} for (C-H). EI Mass (70eV) m/z: showed a molecular ion peak (M+) at 426.7 calculated for C_{30} H_{50} O with characteristic fragment ions at 411 (M+- Me), 393 (M+-Me-H_2O), 365, 299, 297, 245, fragment ions at m/z 220, m/z 207 (allocate the hydroxyl group at C3 position), m/z 218, m/z 205 and m/z 189 all in accordance with the lupene skeleton[4]. The ^{1}HNMR spectrum (400 MHz in $CDCl_3$) in Table 1 exhibited seven tertiary methyl groups and the vinylic methyl at δ 1.69 ppm. In addition, a signal at δ 3.15 with a coupling of ~3 and 6 Hz is indicative of C3-H α-orientation. Two vinylic proton signals at δ 4.59 and 4.70

ppm are indicative of a terminal methylene group at C-20. ^{13}CNMR (100 MHz, in $CDCl_3$) in Table 1 confirmed the chemical shift assignments for lupeol, which were consistent with previously reported data. [4] The ethanol extract of the aerial parts and the main isolate were then tested for their antifungal and nematicidal effects.

Table (1): ^{1}HNMR and ^{13}CNMR (400MHz, and 100 MHz$CDCl_3$) of lupeol

Position	δH ppm	δC ppm
1		38.7
2		27.4
3	3.23 (1H,m)	79.0
4		38.8
5		55.3
6		18.3
7		34.6
8		40.8
9		50.4
10		37.1
11		21.1
12		25.1
13		38.0
14		42.8
15		27.6
16		35.5
17		43.0
18		47.9
19	2.36 (1H,t)	48.3
20		150.9
21		29.9
22		40.0
23	0.78 (3H, s)	28.0
24	0.81 (3H, s)	15.3
25	0.85 (3H, s)	16.1
26	0.96 (3H, s)	15.9
27	0.99 (3H, s)	14.5
28	1.05 (3H, s)	18.0
29	4.59 (1H, br.s, H-29a) 4.70 (1H, br.s, H-29b)	109.3
30	1.70 (3H,s)	19.3

3.2. Antifungal activity

3.2.1 Inhibition of mycelial growth

The results of antifungal screening with various concentrations of EE and L from R. tingitana are presented in Table 2. In this study, EE exhibited a greater reduction in mycelial growth compared to L. All concentrations showed inhibitory activity against the fungus A. flavus. Different concentrations of R. tingitana extract inhibited the growth of A. flavus in vitro, as shown in Table 2. Among them, the concentrations of 100, 110, and 120 ppm were the most effective in reducing the radial growth of the fungus, while the concentration of 10 ppm was the least effective in inhibiting the radial growth of this fungus.

Table (2): Effect of different concentration of crude extract (EE)and Lupeol (L) from *R. tingitana* on the percentage of reduction in fungal growth and spore germination of *Aspergillus flavus*

Plant materials	Concentration (ppm)	Diameter of colony (mm)	%of inhibition (%)	%of germination (%)	(%)Inhibition of spore germination
EE	10	77	7.2	100	0
	20	70	15.7	95	5
	30	65	21.7	87	13
	40	52	37.3	80	20
	50	50	39.8	78	22
	60	45	45.8	68	32
	70	41	50.6	53	47
	80	39	53.0	45	55
	90	37	55.4	37	63
	100	28	66.3	35	65
	110	25	69.9	28	72
	120	19	77.1	22	78
L	10	82	0.0	100	0
	20	81	1.2	100	0
	30	78	4.9	97	3
	40	75	8.5	86	14
	50	68	17.1	81	19
	60	57	30.5	74	26
	70	55	33.0	58	42
	80	53	35.4	48	52
	90	44	46.3	40	60
	100	41	50.0	38	62
	110	37	54.9	32	68
	120	29	64.6	27	73
Control	100	82	0.0	100	0.0

EE: Ethanol extract of the aerial parts of *R.tingitana*,L: Lupeol

3.2.2. On spore germination

The data presented in Table 2 demonstrate a reduction in spore germination of A. flavus due to the inhibitory effects of the two tested samples of R. tingitana (EE and L). The highest proportion of spore germination was observed at 30 ppm of EE and L from R. tingitana, with percentages of 87% and 97%, respectively. Conversely, the lowest percentage of spore germination was observed at 120 ppm of EE and L, with percentages of 22% and 27%, respectively. Lupeol (L) appears to be more effective in inhibiting spore germination than EE.

3.2.3. Inhibition of pod and kernel treatments

The in vivo inhibitory activities of active R. tingitana samples on A. flavus incidence in peanut pods and kernels of the susceptible variety (Giza 3) were determined and the results are presented in Table 3. Inoculation of A. flavus on pods and kernels resulted in 100% infection after 6 days in the absence of treatment. However, when peanut pods and kernels were treated with EE and L of R. tingitana at a concentration of 120 ppm immediately before, during, or after inoculation, A. flavus colonization was completely inhibited.

There was no significant difference observed between the three treatment timings with EE of R. tingitana in terms of the inhibition percentage of A. flavus (100%). On the contrary, the percentage of A. flavus incidence was significantly reduced when treated with lupeol (L) of R. tingitana immediately after inoculation (100%) for both pods and kernels. In the case of lupeol treatment two days before inoculation, the incidence of A. flavus decreased to 90% for both pods and kernels. Similarly, when treated two days after inoculation, the incidence decreased to 80% for pods and 90% for kernels.

Table (3): Effect of different concentrations of crude extract (EE) and Lupeol(L) from *R. tingitana* on the percentage of reduction in *A. flavus incidence*

***R. tingitana* treatments (120ppm)**	**Treatment time**	***A.flavus* incidence (%)**		**Inhibition over control (%)**	
		Pods	**Kernels**	**Pods**	**Kernels**
EE	Immediately after inoculation	0^{d}	0^{c}	100^{a}	100^{a}
	Two days before inoculation	0^{d}	0^{c}	100^{a}	100^{a}
	Two after before inoculation	0^{d}	0^{c}	100^{a}	100^{a}
L	Immediately after inoculation	0^{d}	0^{c}	100^{a}	100^{a}
	Two days before inoculation	10^{c}	10^{b}	90^{b}	90^{b}
	Two days after before inoculation	20^{b}	10^{b}	80^{c}	90^{b}
Without (control)		100^{a}	100^{a}	0^{d}	0^{c}

Means within the same column followed by the same letter are not significantly different according to Duncan's multiple range test (P≥0.05). EE: Ethanol extract of the aerial parts of *R.tingitana* , L: Lupeol

Plants produce a variety of compounds as a defense mechanism against various microorganisms, including plant pathogens and environmental organisms. These compounds are indicative of the effective protection mechanisms developed by plants. Consequently, plants and their secondary metabolites offer a promising source of structurally diverse active compounds, including antimicrobials. [31] Lupeol, a triterpene belonging to the lupane class, has been previously reported to exhibit potent antibacterial and antifungal properties. [4] The ethanol extracts of the aerial parts of R. tingitana showed superior inhibitory activity against the fungus Aspergillus flavus compared to lupeol. This difference in activity may be attributed to the synergistic effect of the secondary metabolite components present in the ethanol extract. In global peanut production, contamination with A. flavus and aflatoxins is a significant challenge, as these mycotoxins are of great concern due to their toxicological impact on humans and animals. [32] Raji and Raveendran [33] have reported that water extracts from Asteraceae members exhibit strong inhibitory effects on the growth of A. niger compared to species from other plant families. They suggest that specific compounds with unknown functional groups present in Asteraceae members may play a role in inhibiting fungal colonies. This observation may explain the fungal inhibitory properties observed in the Asteraceae family member, R. tingitana. In line with this, both EE and L at a concentration of 120 ppm from R. tingitana inhibited A. flavus incidence in peanut pods and kernels.

Extensive research has been conducted to investigate the antifungal activity and potential mechanisms, as well as the in vitro and in vivo anti-aflatoxigenic efficiency, of natural products derived from medicinal plants against A. flavus. [34,35]

3.3. Nematicidal activity

The impact of R. tingitana EE and L extracts at seven concentrations (2.5, 5, 10, 20, 40, 80, and 120 ppm) on the mortality percentage of newly hatched M. incognita and T. semipenetrans juveniles is presented in Table 4. In general, a positive relationship has been observed between nematode juveniles' mortality percentages and extract concentration at three different exposure times. Larval mortality percentages increased with the increase of R. tingitana extract concentration from 2.5 ppm up to 120 ppm. Table 4 also demonstrates that the tested concentrations of EE were superior to those of L in terms of larval mortality percentages at all exposure times, with mortality values of 98.2% and 91.8%, respectively, after 72 hours.

Furthermore, the records in Table 4 illustrate the efficacy of R. tingitana EE and L on the mortality percentage of T. semipenetrans juveniles at the seven concentrations (2.5, 5, 10, 20, 40, 80, and 120 ppm). Both EE and L reduced the activity and induced mortality in the nematode. It is also evident that the tested concentrations of R. tingitana EE were superior to those of L in terms of larval mortality percentages at all exposure times, with the highest mortality (93%) occurring at 120 ppm within 72 hours and the lowest (33.33%) at 2.5 ppm. Conversely, L recorded the highest mortality (88%) at 120 ppm within 72 hours and the lowest (24%) at 2.5 ppm.

Table (4): The nematicidal effects of *R.tingitana* (EE& L) on mortality percentage of ***T. semipenetrans*** **and *M. incognita* juveniles under different concentrations at different exposure times.**

Treatment	Conc.	*T. semipenetrans* %Mortality(J2) / Time			*M. incognita* % Mortality(J2) / Time		
		24h,	48h.	72h.	24h,	48h.	72h.
EE	2.5	29.77	32.22	33.33	31.33	35.33	38.12
	5.0	33.54	39.00	42.77	36.00	41.00	49.67
	10.0	41.66	43.32	49.99	43.77	46.65	58.00
	20.0	42.87	46.00	53.11	45.66	55.76	63.75
	40.0	50.66	53.33	89.65	66.53	72.54	83.87
	80.0	53.22	65.33	91.00	79.55	88.60	92.44
	120.0	69.00	84.33	93.00	80.88	89,92	98.22
L	2.5	20.00	23.07	24.00	25.30	28.62	33.97
	5.0	25.77	26.99	38.23	32.33	35.45	39.93
	10.0	30.76	31.22	40.34	35.43	41.66	55.21
	20.0	32.46	42.00	49.22	45.00	53.55	66.23
	40.0	35.76	45.77	65.33	65.43	69.00	84.00
	80.0	52.08	75.00	84.37	69.77	72.78	88.66
	120.0	63.33	79.34	88.00	75.87	85.55	91.89
Check	00	00	00	00	00	00	00

Conc.:concentration, EE: Ethanol extract of the aerial parts of *R.tingitana* ,L Lupeol.

3.3.1. Efficacy of R.tingitana application on M. incognita development infected eggplant (Solanum melongena) under greenhouse conditions.

Data in Table 5 reveal that R. tingitana EE and L, when applied at rates of 40, 80, and 120 ppm/pot, exhibit bio-nematicidal potential. This potential was compared with the bio-product Micronema, used as a standard bio-nematicide at a dose of 12 ml/pot against Meloidogyne incognita infecting eggplant. The results showed a significant reduction in gall formation, egg masses, the final population, nematode build-up, and egg production per egg mass compared to the untreated control.

Differences in nematode reduction were evident among the treatment doses. The higher the dose, the greater the decrease in nematode numbers, with the maximum dose of 120 ppm/pot yielding the best results. There was a noticeable decrease in nematode juveniles (J2) per soil across all treatments, regardless of the concentration levels.

Table (5): Effect of *R.tingitana* (EE and L) on *M. incognita* development infected eggplant(*Solanum melongena*) under greenhouse conditions.

Treated	Dose /pot	Galls/ root	%R	In soil	%R	Egg masses /root	%R	Eggs/ egg mass	%R	Females	%R	F.P	%R	R.B
EE	120ppm	69^{a}±4.10	88.24	479^{a}±3.48	87.95	18^{a}±1.15	92.82	95^{a}±2.08	80.80	48^{a}±2.08	86.47	640^{a}±5.49	87.39	0.32
	80ppm	96^{b}±5.51	83.64	1114^{e}±7.02	71.98	26^{b}±1.15	89.64	156^{c}±1,00	68.48	61^{b}±1.00	82.81	1357^{d}±12.35	73.27	0.63
	40ppm	157^{de}±10.14	70.18	2117^{g}±16.67	46.67	68^{d}±3,00	72.90	242^{f}±1.45	51.11	98^{d}±1.45	72.39	2525^{f}±15.68	50.26	1.26
L	120ppm	138^{c}±8.37	76.49	727^{b}±14.53	81.71	63^{d}±1.45	74.90	139^{b}±1.35	71.91	117^{e}±1.53	67.04	1045^{b}±18.01	79.41	0.52
	80ppm	179^{e}±2.89	69.50	1312^{f}±6.11	67.01	101^{f}±2,08	59.76	167^{cd}±0.58	66.26	161^{f}±0.58	54.64	1741^{e}±11.46	65.70	0.87
	40ppm	235^{f}±2.79	59.96	2263^{h}±29.63	43.09	121^{g}±3.84	51.79	203^{e}±6.01	58.98	213^{g}±6.01	40.00	2810^{g}±24.53	44.65	1.40
Bio product	12ml	127^{c}±2.87	78.36	853^{d} ±4.26	80.08	76^{e}±186	69.72	175^{d}±2.19		81^{c}±0.88	77.18	1127^{c}±10.99	80.08	56.
Check	-----	587^{g}±18.02	-------	3977^{i} ±14.53	------	251^{b}±1053		495^{g}±3.18		355^{h}±0.88	----	5077^{h}±13.75	-----	2.53
F. value		389.20	------	3766.03	-----	1114.78	-----	805.33	---	526.70	-----	6965.75	------	-----
P. value		00		0.00		0.00		0.00		0.00		000		-----

* All values are the mean of three replicates. Numbers following "±" represent the standard errors (SE). Different letters in the same column indicate statistically significant differences at the 0.05 probability level according to the Duncan test.EE: Ethanol extract of the aerial parts of *R.tingitana* ,L Lupeol,R:reduction,R.B: rate of buildup, F.P:final population.

For instance, both EE and L at the highest concentration level of 120 ppm/pot significantly reduced the percentage of nematode juveniles, with reductions of 87.39% and 81.71%, respectively. The lowest reduction in juvenile count, 46.76% and 43.09%, was obtained at a concentration of 40 ppm, respectively. As the concentration increased, the number of galls progressively decreased. The highest reduction, 88.24% and 76.49%, was observed at a concentration of 120 ppm in both EE and L treatments, respectively, while the lowest reduction was 72.39% and 40.00% at a concentration of 40 ppm.

Counts of egg masses per plant in most treatments were significantly lower than those in the control. The highest percentage reductions in egg mass production, 92.82% and 74.90%, were achieved at a dose of 120 ppm/pot in both EE and L treatments, respectively. Counts of nematode females within the roots of eggplants treated with R. tingitana EE showed that the higher dose resulted in lower female counts. The highest percentage of reduction in females was 86.47%, while the lowest was 72.63%. In the case of the L treatment, the highest concentration resulted in a reduction of 67.04%, and the lowest concentration led to a reduction of 59.84%.

On the other hand, both EE and L suppressed the final nematode population values, with averages of 87.39% and 79.41% at a concentration of 120 ppm, respectively. In comparison, Micronema, used as a standard bio-nematicide at a dose of 12 cm/pot, suppressed the final nematode population values with an average of 77.80%. The calculated rates of build-up revealed a similar trend, with the higher-dose treatments (EE and L) resulting in nematode populations folding by 0.42 and 0.52, respectively, at the highest concentration of 120 ppm, compared to 1.26 and 2.53 folds in the control treatment.

3.3.2. Growth response of M. incognita-infected eggplant plants as influenced by of R.tingitana (EE and L) application

Improvement in plant growth parameters, such as shoot and root length, as well as weights, varied and correlated with R.tingitana EE and L treatments at different doses

(Table 6). The concentration of 120 ppm in the EE treatment resulted in the best outcomes, with significant increases in fresh weight (91.68%), dry weight (81.00%), shoot length (28.21%), and leaf count (40%). In comparison, the L treatment at the same concentration caused increases of 68.75%, 33.33%, 25.64%, and 20%, respectively. On the other hand, the lowest concentration of 40 ppm in the EE treatment led to percentage increases of 31.25%, 31.19%, 10.98%, and 20%. In contrast, the standard bio-nematicide, Micronema, at a dose of 12 cm/pot, showed significant percentage increments in fresh weight (68.75%), dry weight (57.43%), and shoot length (18.33%). Therefore, the EE of R.tingitana exhibited the most significant improvement in plant growth parameters.

Table (6): Plant growth parameters of eggplant (*Solanum melongena*) affected by *Meloidogyne incognita* and treated by *R.tingitana* (EE and L) in different concentration under greenhouse conditions

Treatments	Dose/pot	Shoot								Root			
		Length (cm)	%Increase	Fresh Weight g)	%Increase	Dry Weight (g)	%Increase	Leaves (no)	%Increase	Length(cm)	%Increase	Fresh Weight(g)	%Increase
EE	120ppm	41.33^{d} ±1.86	28.21	30.67^{d}±1.86	91.68	6.23^{d}±0.37	81.63	7.00^{c}±0.33	40.00	22.67^{abc}±1.20	22.05	20.60^{d}±1.40	31.57
	80ppm	35.67^{abcd}±0.33	16.82	25.67^{c}±2.40	60.43	5.50^{cd}±0.35	60.34	6.00^{bc}±0.33	20.00	21.33^{abc}±0.88	17.15	16.67^{c}±0.33	23.99
	40ppm	33.33^{abc}±0.33	10.98	25.00^{c}±0.58	31.25	4.50^{b}±0.15	31.19	6.00^{bc}±0.33	20.00	21.00^{abc}±2.08	15.85	15.33^{c}±0.33	17.35
L	120ppm	39.33^{bcd}±0.67	25.64	27.00^{cd}±2.00	68.75	4.57^{ab}±0.47	33.23	6.00^{bc}±0.58	20.00	25.67^{c}±0.67	31,16	16.00^{c}±0.58	20.81
	80ppm	37.67^{bcd}±1.45	21.23	21.00^{b}±0.58	31.25	4.13^{ab}±0.12	20.40	5.00^{a}±0.58	--	21.33^{abc}±0.67	17.15	15.33^{c}±0.33	17.35
	40ppm	33.33^{abc}±5.51	10.98	19.33^{ab}±0.33	20.81	3.87^{ab}±0.03	12.82	5.00^{a}±0.00	--	19.67^{ab}±3.84	10.16	14.33^{bc}±0.33	11.58
Bio product	12ml	36.33^{abcd}±2.03	18.33	27.00^{cd}±0.58	68.75	5.40^{c}±0.32	57.43	6.00^{ab}±0.00	20.00	24.00^{bc}±1.15	26.37	15.33^{c}± 1.33	17.35
Healthy		30.67^{ab}±2.87	3.26	28.00^{cd}±1.15	75.00	6.07^{cd}±0.12	76.96	7.00^{c}±00	40.00	19.67^{abc}±3.84	10.16	16.00^{c}±.58	20.81
Check	-	29.67^{a}±0.33	-----	16.00^{a}±1.15	----	3.43^{a}±0.07	------	5.00^{ab}±0.33	------	17.67^{a}±0.33	----	12.67^{ab}±0-63 ---	--
F. value	-		-----	35.65	-----	20.62	-----			54.20	-----	46.06	
P. value		0.00	-----	0.00	-----	0.00	-----			0.00	----	0.00	----

All values are the mean of three replicates. Numbers following "±" represent the standard errors (SE). Different letters in the same column indicate statistically significant differences at the 0.05 probability level according to the Duncan test. EE: Ethanol extract of the aerial parts of *R.tingitana* ,L Lupeol.

Additionally, R.tingitana (EE and L) exhibited high toxicity against nematodes in laboratory exposure tests and proved effective in controlling nematode infestations in eggplant roots over a 45-day period. Furthermore, improvements in plant growth parameters, such as shoot and root length and weight, were observed and varied depending on the R.tingitana (EE and L) treatments at different doses. The induction of resistance in susceptible host plants against invading nematodes has been a significant goal in developing nematode management programs. The application of amino acids to infected plants may elevate protein, lipid, and phenol levels [36] thereby activating physiological processes in plants to resist and overcome invading pathogens.

Many plant products associated with resistance responses to nematodes belong to classes of compounds such as alkaloids, glucosides, and organic acids. [37] Some metabolites found in several plant species have been tested for their nematicidal activity. For instance, Tithonia diversifolia (Hemsl.) A. Gray, which is rich in alkaloids, can suppress egg hatching of M.incognita by up to 98% starting from 2 days after incubation. The ethanol extract

of Alstonia scholaris flowers, when subjected to chromatographic examination, yielded several triterpenoid compounds that demonstrated nematicidal effects. [38] Numerous plant species worldwide are known to possess pesticidal properties. The application of botanicals, biological control, and soil amendment approaches is widespread, primarily due to their environmental safety. Several tested plants and phytochemical isolates with reported in vitro or in vivo nematicidal properties have been documented. [39] The mechanisms of action of plant extracts may include denaturing and degrading proteins, inhibiting enzymes, and interfering with electron flow in the respiratory chain or ADP phosphorylation. [40]

4. DISCUSSION

A wide variety of compounds are known to be produced by plants to defend themselves against various microorganisms, including plant pathogens and environmental organisms. These compounds are indicative of the effective protective mechanisms developed by plants. Consequently, plants and their secondary metabolites offer a promising source of structurally diverse active compounds, including potential antimicrobials. [30] Lupeol, as a lupane class triterpene, has been reported to exert potent antibacterial and antifungal properties. [4]

The aerial part ethanol extracts of R. tingitana demonstrated superior inhibitory activity against the fungus Aspergillus flavus compared to lupeol. This difference may be explained by the synergistic effects of the secondary metabolite components present in the ethanol extract. Worldwide peanut production faces a significant challenge due to contamination with A. flavus and aflatoxins, which are of great concern due to their toxicological effects on humans and animals. [31] Raji and Raveendran [32] reported that water extracts of Asteraceae members exhibited the strongest effect in reducing the growth of A. niger compared to species from other families. They suggested that specific compounds with unknown functional groups present in Asteraceae members may play a role in inhibiting fungal colonies. This may explain the antifungal properties observed in the study species of the Asteraceae family, H. radicata. Consistent with these findings, both EE and L at concentrations of 120 ppm from R. tingitana inhibited A. flavus incidence in peanut pods and kernels. The antifungal activity and potential mechanisms, both in vitro and in vivo, of natural products derived from medicinal plants against A. flavus have been extensively investigated. [33,34]

R. tingitana (EE and L) exhibited high toxicity against nematodes in laboratory exposure experiments. Furthermore, they proved effective in controlling nematode infestations in the roots of eggplants over 45 days. The improvement in plant growth parameters, such as shoot and root length and weight, varied and correlated with the doses of R. tingitana (EE and L) treatments. The induction of resistance in susceptible host plants against invading nematodes has been a major goal in the development of nematode management programs. The application of amino acids to infected plants may lead to elevated levels of proteins, lipids, and phenols. [35] These compounds, in turn, activate physiological processes in plants to help them respond to and overcome invading pathogens.

Many plant products associated with resistance responses to nematodes belong to classes of compounds like alkaloids, glucosides, and organic acids. [36] Some of the metabolites found in various plant species have already been tested for their nematicidal activity. For example, Tithonia diversifolia (Hemsl.) A. Gray, which is rich in alkaloids, can suppress egg hatching of M. incognita by 98% within 2 days after incubation. Chromatographic examination of the ethanol extract derived from the flowers of Alstonia scholaris revealed several triterpenoid compounds that demonstrated nematicidal effects. [37] Numerous plant species worldwide are known for their pesticidal properties. The application of botanicals,

biological control methods, and soil amendments, among other strategies, is considered environmentally safe. [41-43] Several tested plants and phytochemical isolates with reported in vitro or in vivo nematicidal properties have been documented. [38] The mechanisms of action of plant extracts may include protein denaturation and degradation, enzyme inhibition, and interference with electron flow in the respiratory chain or with ADP phosphorylation. [39]

5. CONCLUSION

The antifungal and bio-nematicidal effects of the ethanol extract (EE) from the aerial parts of R. tingitana are greater than those of lupeol (L). This difference can be attributed to the synergistic interactions among the various secondary metabolites present in the ethanol extract, which are responsible for the observed activities. Both EE and L have the potential to be used as antifungal and bio-nematicidal agents.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Plant material supplement: OS. Design of the experiment and writing of the article: A M S, S G, GM, AH. Performing the chemical study: A MS. Performing the biological study: G M and AH. All authors have read and approved the manuscript.

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تقييم إمكانات مبيدات الفطريات والنيماتودا في والديدان الخيطية النباتية على الفطريات الممرضة للنبات Reichardia tingitana (L.) Roth

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ملخص

كانت إزالة العديد من مبخرات التربة ومبيدات النيماتودا هي الشغل الشاغل لسلامة الإنسان والبيئة. يمكن أن تسبب مسببات الأمراض الفطرية أمراضًا خطيرة للإنسان والحيوان. تتسبب العقدة العقدية الجذرية، و *Meloidogyne incognita* و *Tylenchulus semipenetrans* في حدوث ضرر كبير وانخفاض في الغلة للعديد من النباتات الاقتصادية. لذلك، كان الهدف من الدراسة هو تقييم أنشطة مبيدات الفطريات ومبيدات النيماتودا لمستخلص الإيثانول (EE) و lupeol (L)؛ عزلة رئيسية للأجزاء الهوائية من *Reichardia tingitana* L. Roth (Asteraceae) على *Aspergillus flavus* والنيماتودا الطفيلية النباتية. تم اختبار التأثيرات المضادة للفطريات لـ EE(10−120 جزء في المليون) و L(23.4− 281.2 ميكرومتر) من خلال مقايسات في المختبر والنمو في الجسم الحي ومقايسات تثبيط إنبات الجراثيم وفعالية تثبيط عدوى القرون والنواة، على التوالي. في حين تم اختبار نشاط مبيد النيماتودا من EEو Lخلال تحضير كتل بيض من أنواع النيماتودا *M.incognita* من الباذنجان المصاب و *T. semipenetrans*من جذور الحمضيات المصابة بتركيز 80،40،20،10،5،2.5 و 120 جزء في المليون (EE) *R. tingitana*. و (L)كانا نيماتوستاتيًا أو مبيدًا للنيماتودا لحيوية الديدان الخيطية، وفقس البيض في المختبر، والتطور والتكاثر في الجسم الحي. يلعب Lupeol دورًا فعالاً في تثبيط استعمار A. flavusفي الفول السوداني. كان EEو Lشديد السمية ضد الديدان الخيطية في التعرض المختبري، كما أنه فعال في السيطرة على إصابة جذور الباذنجان بالديدان الخيطية لمدة 45 يومًا. كان التحسن في معاملات نمو النبات من حيث الفروع وطول الجذور والأوزان متغيرًا ومتناسبًا مع معاملتي EE و L بجرعات مختلفة. تتفوق التأثيرات المضادة للفطريات والمبيدات الحيوية لمستخلص الإيثانول في الجزء الجوي من يمكن استخدام *R.tingitana* على تأثير اللوبيول الذي يمكن تفسيره على أنه التأثير التآزري للمواد الكيميائية النباتية في مستخلص الإيثانول. كعوامل مضادة للفطريات ومبيدات حيوية.

الكلمات الدالة: الأفلاتوكسين، الرشاشيات فلافوس، الشوك الكاذب، اللوبيول، النيماتودا النباتية، الترايتيربين.

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Quantification of Mangiferin from the Bioactive Fraction of Mango Leaves (*Mangifera indica* L.) and Evaluation of Wound-Healing Potential

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ABSTRACT

Burns refer to damage to the skin's surface caused by exposure to high temperatures, which can be due to factors such as oil, water, electricity, fire, sun exposure, and chemicals. Prompt and appropriate treatment is essential to prevent undesirable consequences. Thus, this study aimed to quantify mangiferin, a potential treatment for burns, in the bioactive fraction of mango leaves (Mangifera indica L.) and evaluate its effectiveness in healing burns.The methods employed included thin-layer chromatography (TLC)-densitometry with validation measures, including linearity, detection and quantification limits (LoD and LoQ), precision, accuracy, and quantification. The bioactive fraction was formulated in membranes at concentrations of 5%, 10%, and 15%. These membranes were applied to rabbits previously subjected to six wound burns, and the healing progress was monitored by measuring burn diameter using a vernier caliper every 3 days for a total of 21 days. Mangiferin, the active compound, was detected at a wavelength of 257 nm. Test results yielded a linearity equation, y = 76496x + 2935.7, with a correlation coefficient value of 0.9957, a detection limit of 2.01 µg/mL, a quantification limit of 6.07 µg/mL, a coefficient of variation ranging from 0.59% to 3.33%, and an accuracy range of 99.18% to 100.9%, with mangiferin levels at 208.31 µg/mL. The membrane preparations of the bioactive mangiferin fraction were evaluated on second-degree burns in rabbits, with concentrations of 10% and 15% showing the most effectiveness.

Keywords: bioactive fraction, mangiferin, burns, membrane, quantification.

1. INTRODUCTION

Burns represent a global health challenge with high mortality and morbidity rates, resulting in a minimum of 180,000 deaths annually (1). Furthermore, more than 96% of burn cases occur in low- and middle-income countries (1). According to data from the Health Ministry of Indonesia (2008), the prevalence of burns was reported at 2.2%, contributing to approximately 40% of all deaths (2). Burns have the potential to damage not only the skin but also other critical tissues, including blood vessels, nerves, tendons, and bones (3). They also significantly elevate the risk of infection, which is the primary cause of death in 61% of burn patients (4). This underscores the importance of effectively controlling bacterial infections during burn treatment to substantially reduce mortality. Aerobic bacteria, including Pseudomonas aeruginosa, Staphylococcus aureus, Acinetobacter baumannii, and Klebsiella pneumoniae, are common culprits in burn-related infections (5). Previous studies have revealed the existence of antibiotic-resistant bacteria, such as methicillin-resistant S. aureus (MRSA) (5). Consequently, it is imperative to administer appropriate treatments to burn patients to prevent potentially fatal bacterial infections.

In the exploration of sustainable Sumatran medicinal

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plants and the search for alternative medicines (6, 7), mango leaves (Mangifera indica Linn.) have been reported to have the potential to treat burns. Mango is a tropical plant, and its production volume increases every year. In 2016, Padang City produced 358 tons of mangos, and this volume increased to 1655 tons in 2020 (8); however, the community primarily utilizes only the fruit. Several studies have revealed that the ethanol extract of mango leaves has efficacy as an antifungal, anti-inflammatory, anticancer, antioxidant, and antimicrobial analgesic agent (9-12). The antibacterial activity of mango leaf extract can inhibit the growth of various bacteria, including Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis, Salmonella typhi, and Shigella flexneri, at concentrations ranging from 150 mg/ml to 250 mg/ml, with the effect improving as the concentration increases[13].

The efficacy of mango leaf ethanol extract as an anti-inflammatory, antioxidant, and antimicrobial agent makes it a valuable candidate for burn therapy. From a chemical perspective, it contains various secondary metabolites, with mangiferin being the primary constituent. The structural formula of mangiferin is presented in Figure 1. Several studies have shown that this compound is responsible for the aforementioned pharmacological effects (14). Therefore, the objective of this study is to determine the levels of mangiferin in the bioactive fraction of mango leaves and evaluate its effectiveness in healing burns in rabbits.

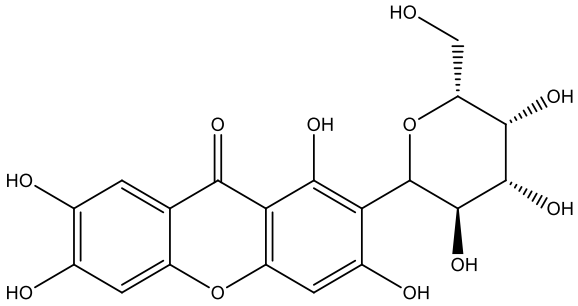

Figure 1. Mangiferin

2. MATERIALS AND METHODS

2.1. Plant material

Mango leaves were collected in 2018 in Padang, West Sumatra, Indonesia. The leaves were identified and authenticated by Dr. Nurainas at the Herbarium Laboratory of the Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, under specimen number 460.

2.2. Instrumentations, Reagents and materials

All chemicals and reagents used were of analytical grade, including methanol (Merck®), ethyl acetate (Merck®), n-hexane (Merck®), formic acid (Merck®), silica gel PF 60 (Merck®), glycerin (Merck®), PVA (Merck®), nipagin (Merck®), nipasol (Merck®), and the reference compound mangiferin (phytopure). The equipment included a UV-Vis spectrophotometer (Shimadzu® UV-1700 PharmaSpec) and a **thin-layer chromatography** (TLC) scanner instrument (Camag®).

2.3. Sample Fractionation

The mango leaves were dried, chopped, and mashed to yield a net weight of 1 kg. Extraction was carried out using the maceration method with methanol as the solvent, involving two immersions for 3 × 24 hours each. During extraction, the mixture was periodically stirred, and the mango leaf powder-to-solvent ratio was 1:20. In the second maceration step, the ratio was adjusted to 1:10. Subsequently,

the extracted liquid was concentrated under vacuum to yield a viscous extract. To further process the extract, it was defatted using the fractionation method with n-hexane, resulting in hexane and methanol fractions. Each fraction was monitored using TLC with an ethyl acetate eluent mixture of formic acid and water (36:6:4) and then compared to pure mangiferin compounds. The methanol fraction was subjected to separation via column chromatography using a step gradient polarity involving n-hexane-ethyl acetate (100:0 → 0:100) and ethyl acetate-methanol (100:0 → 0:100). Each subfraction was re-monitored by TLC and compared to mangiferin to determine the presence of these compounds.

2.4. Quantification and Validation of Mangiferin in the Bioactive Fraction by TLC

The TLC plate used was composed of silica gel 60 F254 with dimensions of 20 × 10 cm. The mobile phase consisted of ethyl acetate: formic acid: water (36:6:4), and the chromatography was conducted in a saturated chamber for approximately 45 minutes. Subsequently, the plate was allowed to air-dry and then quantified using the Camag TLC scanner 4 at a wavelength of 257 nm. The obtained results were analyzed with the winCATS application (version 1.4.7), which generated a linear calibration plot based on the standard regression equation[15].

The TLC-densitometry method was validated using several parameters as described in reference [16,17]:

a) Linearity

Linearity was assessed through data analysis using five standard solution concentrations: 0.05, 0.1, 0.2, 0.3, and ***0.4 mg/mL. Each solution (5 µL) was applied to the same*** plate and eluted using a mobile phase with an ethyl acetate: formic acid: water ratio of 36:6:4, followed by scanning using a TLC scanner at a wavelength of 257 nm.

b) Determination of Limit of Detection (LoD) and Limit of Quantification (LoQ)

The detection and quantification limits were utilized to assess the method's sensitivity. LoD and LoQ values were determined based on linear equations derived from the calibration curve, which was constructed using solutions with concentrations of 0.05, 0.1, 0.2, 0.3, and 0.4 mg/mL. Additionally, the solutions were eluted using the mobile phase ethyl acetate: formic acid: water (36:6:4) and evaluated with a densitometer to calculate the standard deviation (SD) value.

$$LoD = \frac{3.3\ x\ SD}{Slope}$$

$$LoQ = \frac{10\ x\ SD}{Slope}$$

c) Precision

Precision was determined using three concentrations: 0.06, 0.12, and 0.2 mg/mL. The elution process with ethyl acetate: formic acid: water (36:6:4) as the mobile phase was repeated three times. Subsequently, the solutions were scanned using a TLC scanner, and the average standard deviation (SD) and percentage coefficient of variation (CV) were calculated.

$$\%CV = \frac{SD}{AUC} \times 100\%$$

d) Accuracy

Accuracy was assessed as a validation parameter to determine the % recovery, which fell within the range of 98–102%. ***This was achieved by adding 60, 120, and 200*** µg/mL to the samples. The elution process employed an ethyl acetate: formic acid: water ratio of 36:6:4 as the mobile phase. After elution, the mixture was scanned using a TLC scanner, and the percentage recovery value was calculated.

e) Quantification of mangiferin

The mangiferin bioactive fraction was prepared at a concentration of 10 mg in 10 mL. Elucidation was conducted using an ethyl acetate: formic acid: water ratio of 36:6:4 as the mobile phase. The area under the curve (AUC) value was obtained using a TLC scanner, and the mangiferin levels in the bioactive fraction were calculated using a linear regression equation.

2.5. Mangiferin Bioactive Fraction Membrane Formulation

The membrane formulation included various additional substances, such as polyvinyl alcohol (PVA), glycerin, nipagin, nipasol, and sterile water as a solvent. Membranes containing the mangiferin bioactive fraction were formulated at different concentrations, namely 5%, 10%, and 15%, to investigate whether its healing activity exhibited a dose-dependent effect on burn wounds.

2.6. Experimental Animal Protocol

This study received approval from the ethics committee of the Faculty of Medicine, Andalas University, under reference number 302/KEP/FK/2019. Four adult male white rabbits (Oryctolagus cuniculus) with an average weight of 2 kg were housed in the Sumatran Biota Laboratory in individual cages maintained at a room temperature of 26 ± 1°C. They had access to a regular supply of food and water. The rabbits were utilized for experiments 7 days after their arrival at the animal facility. The study adhered to the ARRIVE (Animals in Research: Reporting in Vivo Experiments) criteria for animal experiments.

2.7. Evaluating the Mangiferin Bioactive Fraction Membrane on Experimental Animals

In this study, four adult male white rabbits were included, and they were subjected to six different treatments: no treatment (negative control), treatment with Bioplacenton® (positive control), treatment with a membrane base devoid of the mangiferin bioactive fraction, and treatment with membranes containing 5%, 10%, and 15% mangiferin bioactive fractions. Prior to treatment, the rabbits underwent burns induced by applying hot metal with a diameter of 20 mm. Subsequently, treatments were administered 24 hours after the burns, and the rabbits were observed for a period of **21 days**.

2.8. Data Analysis

The average wound diameter was measured in the vertical, horizontal, and diagonal directions. The percentage of healing was calculated using the following formula:

$$\frac{d_1^2 - d_2^2}{d_1} \times 100\%$$

Note:

d1 = the diameter a day after making the wound (mm)

d2 = the diameter of the wound on the day of observation (mm)

The assessment of wound healing percentage was conducted from the 1st day when the test material was administered up to the 21st day. Data analysis was performed using the two-way analysis of variance (ANOVA) method. A post-hoc Duncan test was employed to determine the impact of the membrane preparations on the percentage of burn wound healing within each group. Results were deemed statistically significant if the p-value was less than 0.05.

3 RESULTS AND DISCUSSION

3.1. Sample Fraction

A total of 1 kg of mango leaves was extracted using methanol as a solvent, with a 6.9% yield. However, the value obtained for the methanolic extract was 16.81%[18]. This was caused by several factors, including altitude, temperature, soil type, and other environmental factors. It was then defatted with n-hexane as a solvent to separate the non-polar phase and produce a methanol extract and an n-hexane fraction from the mango leaf extract. The monitoring of mangiferin in each fraction was carried out via TLC with a mobile phase of ethyl acetate: formic acid: water (36:6:4 v/v) to obtain an Rf value of 0.56, as shown in Figure 2. A previous study used a mobile phase comprising ethyl acetate: distilled water: formic acid (8.5:1.5:1) with an Rf value of 0.66[19]. The production of the methanol fraction from the mango leaves was followed by subfraction separation using vacuum column chromatography with a solvent mixture of n-hexane, ethyl acetate, and methanol, adjusted based on polarity levels. Each fraction was monitored using TLC, resulting in the isolation of 44.9 g of a 100% methanol fraction containing mangiferin

compounds. This product was subsequently formulated into a membrane preparation and tested on experimental animals.

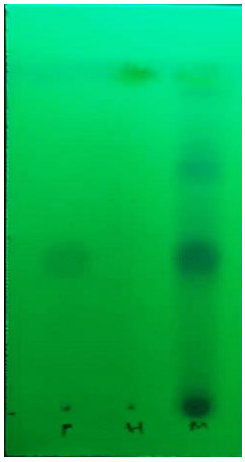

Figure 2. TLC profile of mangiferin with *n*-hexane and methanol extracts with ethyl acetate: formic acid: water (36:6:4) as the eluent, under UV λ_{254} nm (P = pure mangiferin, H = *n*-hexane extract, M = methanol extract)

3.2. Quantification and Validation of Mangiferin in the Bioactive Fraction by TLC-Densitometry

Quantitative analysis is fundamental for providing information about the composition and concentration of secondary metabolites in natural ingredients responsible for specific pharmacological activities. Several analytical methods can be used for quantification, but TLC-densitometry is accurate, simple, and straightforward.[19]

The selective wavelength of mangiferin was obtained at 257 nm, while a wavelength of 258 nm was recorded in another study [20]. The selective wavelength is an identification parameter for mangiferin compounds. The mobile phase used was ethyl acetate: water: formic acid in the ratio of 36:6:4.

3.3. Validation of the Mangiferin Bioactive Fraction

a) Linearity

The linearity analysis was conducted by eluting five concentrations of standard solutions (0.05, 0.1, 0.2, 0.3, and 0.4 mg/mL) on a silica gel 60 F254 TLC plate, followed by the measurement of the area. The calculation yielded the equation y = 76496x + 2935.7 with a correlation coefficient of 0.9957, which falls into the 'fairly good' category, as shown in Figure 3. In contrast, another study obtained a different equation, y = 17,7845x + 194,030, with an R value of 0.9997[19].

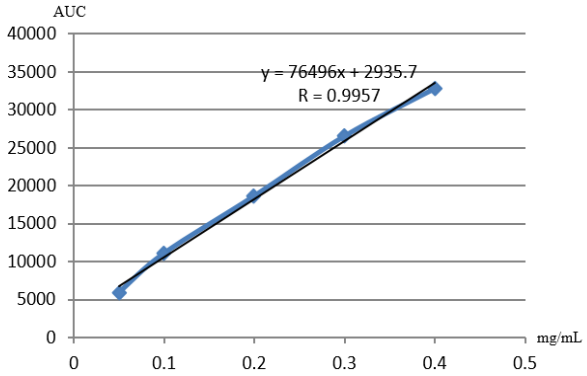

Figure 3. Mangiferin calibration curve

b) LoD and LoQ

The LoD and LoQ values in this study were 2.01 µg/spot and 6.07 µg/spot, respectively. A previous study reported values of 99 ng/spot and 329.8 ng/spot, respectively[19].

c) Precision

The accuracy of this study was analyzed based on the coefficient of variation (CV), which ranged from 0.59% to 3.33%. In another study, the CV value ranged from 0.12% to 0.91%, falling within the required % CV values field, namely % CV < 5%[16,19].

Table 1. Accuracy Test Results

Rate (mg/ml)	SD	% CV
0.06	46.46	0.59
0.12	54.80	1.18
0.2	77.57	3.33

d) Accuracy

The accuracy test was used to determine the proximity of the percentage obtained from the analysis to the actual content of mangiferin. The resulting value was 100.1 ± 0.49% (w/w), falling within the required range of 80–120%[16].

Table 2. Accuracy Value

Actual rate (µg/mL)	Rate earned (µg/mL)	% Recovery
60	60.56	100.9
120	119.02	99.18
200	200.42	100.21

e) Quantification of mangiferin

The quantification of mangiferin in the extract resulted in a value of 208.31 µg/mL, with a yield of 0.94%.

3.4. Mangiferin Bioactive Fraction Membrane Formulation

The evaluation of the mangiferin bioactive fraction membrane preparation formula included visual testing on a white background for colored membranes and on black for colorless ones, as well as testing for homogeneity and thickness. These tests were conducted from the initial gel preparation stage until the formation of the membrane preparation[21].

Based on the results obtained, all membranes received a (++) rating due to their translucent appearance when observed against a white background. Appearance was categorized as follows: (+) cloudy, (++) translucent, and (+++) transparent. The base formula membrane was clear, while the formula membrane containing the bioactive extract exhibited a brown color that intensified with increasing concentrations of the mangiferin bioactive fraction, resulting in a brownish appearance (Fig. 4). Additionally, no lumps were formed, and the preparations were homogeneous[22,23].

The thickness test of the mangiferin bioactive fraction membrane aimed to assess the uniformity of thickness, which is indicative of homogeneity when poured into the mold. Non-uniformity in the material can affect the product's performance. One membrane was tested by measuring it at three different points with a screw micrometer. The examination revealed that the product had an average thickness of 0.19 ± 0.025 mm. Additionally, the results demonstrated that the membrane thickness increased with the concentration of the bioactive fraction of mangiferin. A test for the presence of air bubbles was also conducted, yielding positive results for all products[21,24].

Figure 4. Mangiferin Bioactive Fraction Membrane with concentration: a. 0%, b. 5%, c. 10%, and d. 15%

3.5. Evaluating Membrane Activity on Burns

The activity of membrane preparations containing the bioactive fraction of mangiferin was tested on white male rabbits (Oryctolagus cuniculus). This study aimed to evaluate

the membranes' ability to accelerate the healing of burns on the rabbits' back skin, using concentrations of 5%, 10%, and 15%. Their effectiveness was also compared with that of the standard Bioplacenton, known for its antibacterial activity in wound healing. The study assessed the burn diameter[25].

Burn healing is a complex physiological process in which damaged skin tissue returns to its normal anatomical state following thermal injury. During the healing process, keratinocytes and epidermal cells from the periphery of the damaged tissue proliferate, reducing the area of the injury. In this study, burn healing was assessed through observations after superficial burns were induced in four male rabbits, with an average diameter of 21.49 mm. The membrane preparation was administered to the test animals once every three days, while Bioplacenton was administered once daily[26,27].

Observation of the wound area in all groups over the 21-day period revealed changes in wound size. The period from days 0 to 6 corresponded to the inflammatory phase, while days 6 to 21 marked the proliferation stage, involving the repair of injured tissue[27-29].

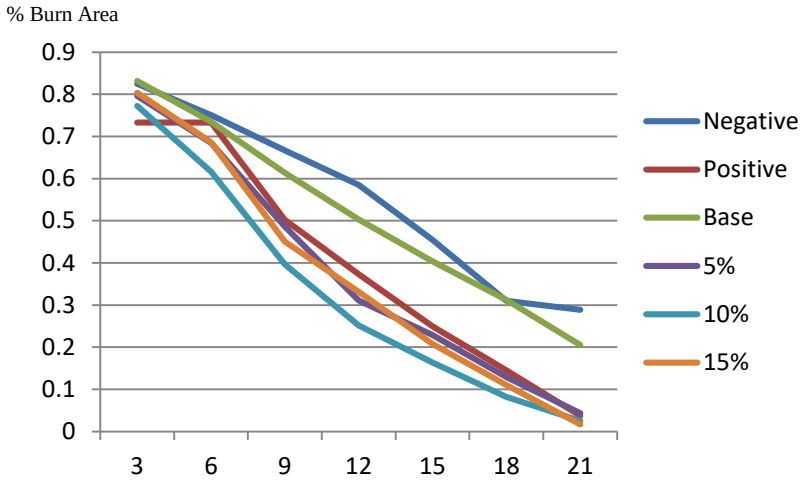

Figure 5. Graph of the percentage of burn area

Figure 5 illustrates the burn-healing process for each treatment from the 1st to the 21st day, showing reductions in the surface area of the burns. On the 21st day, the average percentage of burn healing for each treatment was calculated: the negative control, positive control, and membrane base had values of 71.12%±0.03, 96.23%±0.06, and 79.48%±0.01, respectively. Additionally, the percentage recovery values for the 5%, 10%, and 15% membranes were 95.62%±0.03, 97.44%±0.04, and 98.32%±0.03, respectively. These results indicate that the bioactive fraction of mangiferin from mango leaves has the potential to accelerate burn healing, consistent with several reports highlighting the potency of natural ingredient extracts in wound healing and therapy[30].

The healing effects of burns attributed to the bioactive fraction of mango leaves, including mangiferin, can be attributed to its anti-inflammatory, analgesic, and antibacterial properties. The anti-inflammatory activity is mediated by inhibiting COX-1, COX-2, and PGE-2 production, as well as inactivating the NLRP3 inflammasome [31,32]. The proliferation and maturation phases of burn healing are influenced by factors such as the type and extent of damage, the patient's overall health, and the tissue's regenerative capacity. The intervention of

the mangiferin bioactive fraction membrane plays a crucial role in initiating and facilitating this process. While all concentrations exhibited a healing effect, the 10% concentration proved to be the most potent, as evidenced by the significant reduction in burn size observed from the initial assessment until day 21[33,34].

3.6. ANOVA

The analysis revealed that the dataset consisted of normally distributed data, which included both homogeneous and non-homogeneous subsets. The assessment of normality using the Kolmogorov-Smirnov test indicated that the healing activity of the mangiferin bioactive fraction membrane followed a normal distribution, with a significance level of 0.2, which is greater than the threshold of 0.05. Additionally, the homogeneity test, as determined by the Levene statistical test, indicated that the data were homogeneous, with a significance level of 0.096, also greater than the 0.05 threshold.[35]

A two-way ANOVA comparing all test groups in terms of the percentage of burn wound healing demonstrated a significant effect within the treatment group. Additionally, Duncan's posthoc test indicated significant differences between most groups, except for the comparison between the 5% preparation and the positive control, as well as between the 10% and 15% preparations within the same subset. It was observed that burn healing improved consistently with each passing day[35].

Based on these results, the groups with preparations of 10% to 15% demonstrated the highest effectiveness and exhibited similar activity. However, they showed significant differences when compared to the 5% concentration preparation. Moreover, these higher concentrations offered several advantages compared to the comparator preparation35. Economically, a preparation with a 10% concentration would be more favorable.

4 CONCLUSIONS

Applying a bioactive fraction membrane with a mangiferin content of 208.31 µg/mL and concentrations of 5%, 10%, and 15% significantly affected the healing of superficial second-degree burns in rabbits, resulting in healing percentages of 95.62%±0.03, 97.44%±0.04, and 98.32%±0.03, respectively. Furthermore, a significant difference in healing time was observed among the treatment groups. Membranes with a concentration of 10% to 15% of the mangiferin bioactive fraction were the most effective, achieving percentages of 97.44%±0.03 and 98.32%±0.03 within 21 days.

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القياس الكمي للمانجيفيرين من كسر المنشط الحيوي من أوراق المانجو (منجيفيرا هندية ألـ.) وتقييم إمكانات التئام الجروح

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ملخص

تشير الحروق إلى الأضرار التي تلحق بسطح الجلد بسبب ارتفاع درجات الحرارة من الزيت والماء والكهرباء والنار والتعرض لأشعة الشمس والمواد الكيميائية. تتطلب علاجا سريعا ومناسبا لتجنب الآثار غير المرغوب فيها. لذلك، تهدف هذه الدراسة إلى تحديد كمية المانجيفيرين، التي يمكن أن تعالج الحروق، في كسر المنشط الحيوي من أوراق المانجو (منجيفيرا هندية ألـ.) وتقييم نشاطها في التئام الحروق. تشمل الطرق المستخدمة في قياس إستشراب الطبقة الرقيقة (تي ألـ سي – - TLC) مكثافية بصرية مع التحقق من ضبط الخطية ، وحدود الكشف والقياس الكمي (ألـ أو دي LoD و ألـ أو قيو LoQ) والدقة والتقدير الكمي. تمت صياغة كسر المنشط الحيوي في غشاء بتركيز 5 / 10 و 15%. تم تطبيق الغشاء على الأرانب التي سبق أن تعرضت لحروق 6 جروح وعولجت ضد 4 ذكور من الأرانب. تم قياس تقدم الشفاء بقطر الحروق باستخدام المسماك المورن في كل 3 أيام إلى 21 يوما. تم الكشف عن المركب النشط (المانجيفيرين) بطول موجة 257 نانومتر. أنتجت نتائج الاختبار المعادلة الخطية واي = 2935.7 + x76596 مع قيمة معامل الارتباط 0.9957 ، حد الكشف 2.01 ميكروغرام / مل µg/mL، قيمة حد التقدير 6.07 ميكروغرام / مل، معامل الاختلاف في المدى 0.59 – 3.33 %، ومدى دقة 99.18 – 100.9% مع مستويات المانجيفيرين 208.31 ميكروغرام / مل. تم اختباراستعدادات غشاء كسر المنشط الحيوي المانجيفيرين على حروق من الدرجة الثانية في الأرانب. أظهرت النتائج أن التراكيز 10% و 15% كانت الأكثر فعالية.

الكلمات الدالة: كسر المنشط الحيوي، المنجفيرين، الحروق، الغشاء، القياس الكمي.

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Evaluation of Pharmacotherapy Standards During Pregnancy Among Jordanian Pharmacy Colleges Graduates

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ABSTRACT

Background: Pharmacists' knowledge of medication risks and benefits during pregnancy, as well as their competence in making appropriate therapeutic decisions to optimize medication use among pregnant women, is crucial. This study aims to evaluate the knowledge of Jordanian pharmacists regarding medication risks and safety during pregnancy and assess their abilities to make appropriate therapeutic decisions and optimize medication use.

Methods: A self-administered questionnaire was sent to 400 randomly selected pharmacists practicing in Amman, Jordan. A validated questionnaire, consisting of six sections with predefined options, was employed.

Results: A total of 233 pharmacists completed the questionnaire, resulting in a response rate of 58.2%. Nearly 73.4% of pharmacists (N = 171) correctly identified the drug of choice for hypertension during pregnancy. Over 70% of pharmacists (N = 169) determined the correct dose of aspirin to prevent preeclampsia. About 50% of pharmacists exhibited limited knowledge regarding drug risks and safety during pregnancy. There was a significant difference in the pharmacists' scores on all tests based on their marital status and years of experience ($p = 0.04$ and $p = 0.01$, respectively). Among pharmacists, 79.8% stated that they studied pharmacotherapy during pregnancy in their undergraduate courses.

Conclusion: Pharmacists have demonstrated an inadequate level of preparedness in providing appropriate pharmaceutical care for pregnant women. Therefore, there is an urgent need to collaborate between national health authorities and academic institutions to empower pharmacists and enhance their knowledge and skills necessary to improve the health outcomes of pregnant women.

Keywords: Pregnancy, Drug Therapy, Health Safety, Pharmaceutical Care.

INTRODUCTION

Pharmacists play a crucial role in optimizing medication use during pregnancy. They serve as the first point of contact for patients and are often the final healthcare professionals patients encounter after medications have been prescribed. Pharmacists possess the necessary pharmacotherapy knowledge, skills, and competencies to provide optimal patient care[1]. They are frequently called upon to make therapeutic decisions that require expertise and knowledge to optimize drug therapy for individual patients, including pregnant women.

The use of medications during pregnancy is often necessary, especially for pregnant women with underlying medical conditions. Research has shown that prenatal exposure to certain drugs can pose genuine risks to the fetus, leading to teratogenic effects or neurodevelopmental disorders in offspring[2]. Pharmacists can play a vital role in preventing drug-related adverse effects by assessing the likelihood of fetal exposure, reviewing prescriptions for

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appropriateness, and identifying drug therapy-related problems[1]. Furthermore, pharmacists are frequently consulted by other healthcare professionals due to their specialized expertise in drug therapies. This is also the case when it comes to medication use during pregnancy. Therefore, their level of professional confidence and knowledge regarding the use of drugs during pregnancy are pivotal factors in achieving the best possible outcomes for pregnant women[3, 4].

In today's dynamic healthcare landscape, pharmacists are shouldering greater responsibilities and commitments to enhance their practice[5, 6, 7]. There are growing global concerns about the mismatch between current pharmacy practice and pharmacy education[8]. In developing countries, including the Middle East, pharmacy education and research have often lagged behind the evolving roles of pharmacists[9]. Nevertheless, significant strides have been made in recent years, with changes introduced to pharmacy education at academic institutions in various countries, including Jordan[10]. For pharmacy educators, the practical challenge lies in harnessing this expanding knowledge base to benefit real-world practice[11, 12]. This study aims to evaluate the knowledge of Jordanian pharmacists regarding medication risks and safety during pregnancy, as well as their capacity to make informed therapeutic decisions and optimize medication use.

METHODS

Compliance with ethical standards

Ethical approval was obtained from the Institutional Review Board (IRB) at the Faculty of Pharmacy, Applied Science Private University (approval number: 2022-PHA-26). Electronic consent was obtained from all participants, and their anonymity was maintained as no names were recorded**.**

Study Design and Sample

This observational, descriptive, cross-sectional study was conducted from August to November 2022 among Jordanian pharmacists practicing in Amman. A random sampling approach was used to select participants. Pharmacists practicing outside Amman or those who did not graduate from Jordanian universities were excluded. The study's principal investigator initially contacted potential participants by phone, introduced the study's aim, assured them of its anonymity, and invited them to participate. Pharmacists who agreed to take part in the study were provided with a link to the self-administered questionnaire.

Sample Size Calculation

A list of practicing Jordanian pharmacists in Amman, including their full names, phone numbers, and occupations, was obtained from the national health authority. Microsoft Excel was used to randomly shuffle the list using the RAND function. The online Raosoft ® calculator was then employed to determine the required sample size for the study. It was assumed that 50% of Jordanian pharmacists working in Amman (out of 28,000) possess adequate pharmacotherapy knowledge during pregnancy. The sample size was calculated with a 95% confidence interval and an absolute precision of 5%. Consequently, the calculated sample size for this study was 375.

Questionnaire Development and Structure

After conducting an extensive literature review, the research team developed a questionnaire to assess fundamental pharmacotherapy knowledge necessary for optimizing medication use and safety during pregnancy.

The questionnaire consisted of five sections. The first section collected participants' demographic information and characteristics, including gender, age, marital status, university of graduation, practice setting, and years of professional experience. In this section, participants were also asked if they received official lessons covering pharmacotherapy during pregnancy during their academic studies at the university.

The second section aimed to assess participants' knowledge about medication safety during pregnancy. This section listed twelve medications, and participants were asked to specify the safety of each medication during

different pregnancy trimesters. Participants selected from five predefined options for each medication: safe during the first trimester, safe during the second trimester, safe during the third trimester, safe during all trimesters, and not safe during pregnancy. The third section measured participants' knowledge about medication risks during pregnancy. This section included six questions or statements to identify potential risks associated with medications during pregnancy.

The fourth section presented six clinical cases with predefined options to assess participants' ability to make appropriate therapeutic decisions for pregnant women. These cases depicted pregnant women with various medical conditions or complaints, and participants were tasked with selecting suitable medications for each case. The fifth section evaluated participants' ability to optimize medication use during pregnancy. It included six clinical cases of pregnant women experiencing chronic medical conditions or minor ailments. Participants were asked to choose an appropriate medication or action to optimize drug therapy for each corresponding case.

The face and content validity of the questionnaire was the questionnaire underwent face and content validity assessment by an expert panel, consisting of two academic researchers in pharmacy practice, two community pharmacists, and one practicing gynecologist. The experts provided feedback on the wording, clarity, comprehensiveness, and relevance of each questionnaire item to the study's aim and objectives. Amendments to the questionnaire were made based on their input. Scores were calculated based on the number of correct answers.

Data Analysis

The data were entered into SPSS version 23 for Windows (SPSS) for analysis. Descriptive statistics were used, and results were expressed as numbers and percentages with a mean (± SD). One-way ANOVA tests were employed to determine associations between variables when appropriate. A two-tailed t-test analysis within subjects was conducted to assess the significance of each measured quality attribute. The significance level was set at a P-value ≤ 0.05.

RESULTS

Pharmacists' Characteristics

Out of the 400 pharmacists contacted, 233 completed the questionnaire, resulting in a response rate of 58.25%. Table 1 presents the demographics and characteristics of the participants. Over half of the participants were young, aged between 23 and 28 years (n = 135, 57.9%), and nearly three-quarters were female (n = 164, 70.4%). Approximately half of the participants graduated from a private university in Jordan (n = 122, 52.3%), and the majority worked in community pharmacies (n = 191, 82%).

More than three-quarters of the participants (n = 186, 79.8%) reported receiving official lessons on pharmacotherapy during pregnancy at their university during their academic studies.

Table 1. Participants' Demographics and Characteristics (N = 233)

Age (years)	n (%)
23 - 28	135 (57.9)
29 - 34	52 (22.3)
35 - 40	27 (11.6)
> 40	19 (8.2)
Gender	
Male	69 (29.6)
Female	164 (70.4)
Marital status	
Single	147 (63.1)
Married	80 (34.3)
Divorced	6 (2.6)
University of graduation	
Public university	111 (47.6)
Private university	122 (52.3)
Practice Setting	
Community pharmacy	191 (82.0)
Hospital pharmacy	17 (7.3)
Academia	16 (6.9)
Pharmaceutical sales	9 (3.9)
Years of professional experience	
Less than 5 years	146 (62.6)
5- 9 years	53 (22.8)
10-15 years	15 (6.4)
More than 15 years	19 (8.2)

Pharmacists' Knowledge of Medications Safety during Pregnancy

The assessment of pharmacists' knowledge regarding medication safety during pregnancy is detailed in Table 2. A majority of the respondents (n=207, 88.8%) were unaware that NSAIDs are considered unsafe during the first trimester. However, only 10.7% and 20.2% recognized that pseudoephedrine and systemic glucocorticoids could be used in the second and third trimesters, respectively. None of the participants were aware of the contraindication of intranasal triamcinolone during pregnancy, and only half of them knew that mineral oil (n=114, 49.4%) and simvastatin (n=121, 51.9%) should be avoided during pregnancy.

Table 2. Assessing the Pharmacists' Knowledge about Medications Safety during Pregnancy (N = 233)

	Medication	Safe during the first trimester	Safe during the second trimester	Safe during the third trimester	Safe during all trimesters	Not safe during pregnancy	Number of correct answers n (%)	Number of incorrect answers n (%)
1	NSAIDs	√					26 (11.2)	207 (88.8)
2	Loratadine				√		99 (42.5)	134 (57.5)
3	Pseudoephedrine		√	√			25 (10.7)	207 (88.8)
4	Intranasal triamcinolone					√	0.0 (0.0)	233 (100)
5	Mineral oil					√	114 (49.4)	118 (50.6)
6	Inhaled albuterol				√		55 (23.6)	178 (76.4)
7	Inhaled budesonide				√		53 (22.7)	180 (77.3)
8	Systemic glucocorticoids		√	√			47 (20.2)	186 (79.8)
9	Insulin preparations				√		126 (54.1)	107 (45.9)
10	Nifedipine				√		52 (22.3)	181 (77.7)
11	Simvastatin					√	121 (51.9)	112 (48.1)
12	Methyldopa				√		159 (68.2)	74 (31.8)

Note: Correct answers are indicated by a checkmark.

Pharmacists' Knowledge about Medication Risks during Pregnancy

Table 3 documents the participants' responses to questions assessing their knowledge of medication risks during pregnancy. Among analgesics, only 7.7% of the participants identified that NSAIDs and aspirin can cause premature closure of the fetal ductus arteriosus if used after 30 weeks of gestation (n = 18, 7.7%). Most participants were unaware that the use of triamcinolone spray is associated with fetal malformations of the respiratory system (n=160, 66.7%), and that proton pump inhibitors are among the acid-suppressant medications that commonly increase the risk of gastric infections (n=172, 73.8%). A significant proportion of respondents did not recognize the harmful effect of systemic glucocorticoids and their association with fetal cleft palate (n = 149, 63.9%), as well as the association of angiotensin-converting-enzyme (ACE) inhibitors with neonatal renal failure (n = 153, 65.7%).

Table 3. Assessing the Pharmacists' Knowledge about Medications' Risks during Pregnancy (N = 233)

	Questions or statements	Answers [a]	One correct answer n (%)	Incorrect answer n (%)	Multiple correct answers n (%)
1	Which of the following drugs may cause premature closure of the ductus arteriosus of the fetus if used after 30 weeks of gestation?	- Muscle relaxants - Paracetamol - ***NSAIDs*** - ***Aspirin***	100 (42.9)	114 (48.9)	18 (7.7)
2	Fetus malformations of the respiratory system is linked to the use of the following nasal spray:	- ***Triamcinolone*** - Budesonide - Fluticasone - Mometasone	73 (31.3)	160 (66.7)	
3	Which of the following acid suppressant medications is more likely to cause an increase in gastric pH and hence increases the risk of enteric infections in pregnant women?	- Antacids - H2-blockers - ***Proton pump inhibitors (PPIs)*** - Potassium-competitive acid blockers	61 (26.2)	172 (73.8)	
4	Which of the following medications can cause fetal cleft palate if taken during the 1st trimester of pregnancy?	- Montelukast - ***Systemic glucocorticoids*** - Inhaled albuterol - Inhaled budesonide	84 (36.1)	149 (63.9)	
5	The risks of uncontrolled diabetes mellitus in pregnancy include:	- Neural tube defects - ***Preeclampsia*** - ***Macrosomia*** - Neonatal hyperglycemia	118 (50.6)	53 (22.7)	62 (26.7)
6	Neonatal renal failure is linked to the use of the following medication during pregnancy:	- ***Angiotensin-converting-enzyme (ACE) inhibitors*** - Beta blockers -Thiazide diuretics - Loop diuretics	80 (34.3)	153 (65.7)	

[a] The correct answers are presented in bold and italics corresponding to each question.

Pharmacists' Therapeutic Decision-making Skills

Table 4 presents the cases used to evaluate the participants' skills in therapeutic decision-making. Only half of the participants correctly identified the appropriate medications or herbal remedies for controlling nausea during pregnancy (n = 123, 52.8%). More than half of them recognized that oral cetirizine is the preferred choice for managing allergic rhinitis during the first trimester (n = 127, 54.5%). The majority of participants correctly identified the preferred medications for treating hypertension (n = 171, 73.4%) and dyslipidemia (n = 151, 64.8%) during pregnancy.

Table 4. Cases Assessing the Pharmacists' Therapeutic Decision-making Skills (N = 233)

	Cases	Answers [a]	One correct answer n (%)	Incorrect answer n (%)	Multiple correct answers n (%)
1	A pregnant woman complaining of nausea during her first month of pregnancy. What would you advise her to take?	- ***Oral ginger*** - ***Oral pyridoxine*** - Oral promethazine - Oral diphenhydramine	123 (52.8)	22 (9.4)	88 (37.8)
2	What do you advise a pregnant woman to take, during her 1st trimester, complaining of allergic rhinitis?	- Olopatadine nasal sprays -Triamcinolone nasal sprays - ***Oral cetirizine*** - Oral pseudoephedrine	127 (54.5)	106 (45.5)	
3	A pregnant woman has been suffering from acute diarrhea for the past few days. What would you advise her to do?	- Take Loperamide - Take Bismuth subsalicylate - Take Diphenoxylate with atropine - ***Take fluids to prevent dehydration and initiate probiotics***	184 (79)	49 (21)	
4	A pregnant woman, in her 1st trimester, complaining of constipation. What would you advise her to take?	- ***Oral polyethylene glycol*** - Oral bisacodyl - Oral mineral oil - Oral castor oil	113 (48.5)	120 (51.5)	
5	A pregnant woman presented with gestational hypertension, who was previously on enalapril before getting pregnant. What would you suggest to do to keep her blood pressure controlled during pregnancy?	- Continue enalapril - ***Discontinue enalapril & start nifedipine*** - ***Discontinue enalapril &start methyl dopa*** - Discontinue enalapril & start metoprolol	171 (73.4)	41 (17.6)	21 (9)
6	A pregnant woman suffering from dyslipidemia, is not responding to therapeutic lifestyle changes. Which of the following is (are) appropriate to manage her condition?	- Oral Simvastatin - ***Oral cholestyramine*** - ***Oral omega-3 fatty acids*** - Oral fenofibrate	151 (64.8)	67 (28.8)	15 (6.4)

[a] The correct answers are presented in bold and italics corresponding to each question.

Pharmacists' Ability to Optimize the Medications' Use during Pregnancy

Table 5 presents the cases used to evaluate the participants' ability to optimize medication use during pregnancy. More than half of the participants correctly identified the timing of prandial insulin administration (n

= 138, 59.2%) and the recommended starting dose of fluticasone propionate inhaler in pregnant women with asthma (n = 120, 51.5%). Three-quarters of the participants correctly suggested the aspirin dose for patients at high risk of preeclampsia (n = 169, 72.5%), and 65.7% correctly recommended the appropriate administration of antacids when taking iron supplements to prevent drug-drug interactions (n = 153, 65.7%).

Table 5. Cases Assessing the Pharmacists' Ability to Optimize the Medications' Use during Pregnancy (N = 233)

	Cases	Answers [a]	Correct answer n (%)	Incorrect answer n (%)
1	A pregnant woman with type two diabetes mellitus was on glyburide prior to pregnancy. The physician decided to replace glyburide with a prandial insulin lispro. When should she take this insulin with regard to meals?	- ***Lispro should be injected 15 minutes before meals*** - Lispro should be injected within 15 minutes after meals - Lispro should be injected between meals - Insulin lispro should be injected at any time	138 (59.2)	95 (40.8)
2	What is the optimal duration for using intranasal oxymetazoline for a pregnant woman complaining of severe nasal congestion?	- ***3 days or less*** - 6 days - 7 days - Not recommended during pregnancy	52 (22.3)	181 (77.7)
3	The doctor decided to prescribe a fluticasone propionate meter dose inhaler for a pregnant asthmatic patient. What is the recommended starting dose for this patient?	- ***100-250 mcg*** - 250-500mcg - >500 mcg	120 (51.5)	113 (48.5)
4	What would suggest for a pregnant woman in the last trimester complaining of a severe headache?	- Oral paracetamol - Oral NSAIDs - ***Refer her to a physician*** -Rest, reassurance, and massage	33 (14.2)	200 (85.5)
5	What would you suggest for a pregnant woman with gestational hypertension at high risk of preeclampsia?	- ***Aspirin 80 mg*** - Aspirin 200 mg - Aspirin 300 mg - Aspirin 500 mg	169 (72.5)	64 (27.5)
6	A pregnant woman has been suffering for several days from heartburn after meal intake and asks for your advice. What would you suggest, in addition to lifestyle modifications and dietary changes, knowing that she is already receiving an iron supplement?	- Take antacids and an iron supplement after meals - ***Take the iron supplement at least 2 hours before an antacid and not less than 6 hours after.*** - Take antacids andiron supplement before meals. - Discontinue the iron supplement until the heartburn problem resolves.	153 (65.7)	80 (34.3)

[a] The correct answers are presented in bold and italics corresponding to each question.

Variables Associated with Answering the Test

The results of the univariate analyses are summarized in Table 6. Single participants (mean = 16.58) had significantly higher scores of correct answers on the entire test compared to married participants (mean = 14.79, p = 0.04). Additionally, participants with 10 years of experience or less achieved a higher test score (mean = 17.22 ± 5.86, P = 0.01).

Table 6. Variables Associated with Answering the Test

Gender		**T-test**
Male/Female (Mean)	15.56/16.07	p = 0.58
Age **Mean ± SD** **18 – 23** **23 – 28** **29 – 34** **35 – 40** **> 40**	 15.92±6.43 13.28± 6.73 16.19±6.74 16.94 ± 6.74 15.55 ± 6.75 14.94± 6.70	**One-way ANOVA** P = 0.23
Marital status		**T-test**
Single /Married (Mean)	16.58/14.79	p = 0.04*
Occupation **Mean ± SD**	15.92 ± 6.43	**One-way ANOVA** p = 0.10
University		**T-test**
Public/ Private (Mean)	16.01/15.83	p = 0.83
Experience (Mean ± SD)		**One-way ANOVA**
< 5 years	16.11 ± 6.26	p = 0.01*
5-10 years	17.22 ± 5.86	p = 0.01*
10-15 years	13.26 ± 5.54	p = 0.92
< 15 years	16.05± 5.88	p = 0.13
***P< 0.05 → statistically significant**		

DISCUSSION

Despite the fact that pharmacists do not provide direct obstetric services, they are readily accessible to women at any point during the continuum of care for preventive advice, prescription filling, and the management of minor ailments. Pharmacists have a considerable influence in optimizing patients' drug therapies; however, they are sometimes ill-equipped to provide adequate pharmaceutical care services for the well-being of mothers[13]. The present study was conducted to assess the preparedness of Jordanian pharmacists in providing appropriate patient care to pregnant women.

The current study revealed that pharmacists have inadequate knowledge about the safety of medications used during pregnancy. Most of the participants could not recognize the safety of various medications during the three pregnancy trimesters. Additionally, almost half of the participants could not identify medications contraindicated during pregnancy. These findings were consistent with other studies, where pharmacists exhibited a low level of knowledge about medication safety[14-16]. As such, these findings reflect that pharmacists have insufficient competence to provide patients and healthcare providers with adequate information about the safety of medications during pregnancy.

We also assessed the participants' knowledge about medication risks during pregnancy. Pharmacists must cautiously evaluate the potential risks of medication use versus the risks of untreated disease during pregnancy[3]. Pharmacists should provide patients with information regarding both the benefits and risks of medication use while discussing the limitations of the available information[17]. The

knowledge of pharmacists about the risks of different medications used during pregnancy differs according to the type of medication. Many participants did not identify the risks associated with the use of commonly dispensed prescribed-only medications (POM) and over-the-counter (OTC) medications. Therefore, pharmacists are not well-equipped with adequate knowledge about medications' safety and risks during pregnancy. This underscores the need to incorporate core courses in the pharmacy curriculum among Jordanian pharmacy colleges to adequately cover the pharmacology of commonly used medications and pharmacotherapeutics during pregnancy.

A high proportion of participants did not recognize the medications required to relieve nausea associated with pregnancy. Our results were not in line with relevant studies[18, 19, 20, 21], which could be explained by the limited national availability of OTC nausea medications, potentially hindering the process of selecting the right product. Furthermore, only half of the participants were aware that oral cetirizine is the drug of choice to manage allergic rhinitis, consistent with a previous French study[22]. However, the majority of pharmacists recommended fluid resuscitation to prevent dehydration and the use of probiotics to treat diarrhea, which was consistent with other studies[23, 24]. Most of the participants recognized the drug of choice to treat hypertension and dyslipidemia, which was similar to other relevant studies[25-28]. On the other hand, the pharmacists' responses to the treatment of constipation were inappropriate. Only half of the pharmacists considered oral polyethylene glycol as the drug of choice. A similar finding was retrieved from a Kuwaiti study, in which the participants recommended stimulant laxatives for pregnant women with diarrhea[24]. Thus, our results have demonstrated the inadequate preparedness of Jordanian pharmacists to make appropriate therapeutic decisions for pregnant women.

Pharmacists play a pivotal role in optimizing medication therapy during pregnancy. For example, they are essential in ensuring the safe and effective use of insulin pens in gestational diabetes. Pharmacists monitor patients' response to insulin therapy, ensure glycemic control, provide counseling on the appropriate administration and titration of insulin, and help prevent hypoglycemic episodes[29, 30, 31]. However, about 40% of the participants did not identify the appropriate administration of prandial insulin in relation to meal intake. This raises concerns that pharmacists may not be providing adequate care for general diabetic patients on insulin therapy. Furthermore, less than one-fourth of the participants correctly identified the recommended duration for the use of topical intranasal decongestants containing oxymetazoline. As a result, pharmacists may not be providing appropriate care for patients with minor ailments and could be putting patients at risk of developing rebound congestion[32]. Headaches during pregnancy can be either primary or secondary, with the latter potentially indicating a life-threatening condition. The most common secondary headaches in pregnancy include stroke, cerebral venous thrombosis, eclampsia, and preeclampsia, especially in the third trimester[33, 34]. Remarkably, the majority of the study participants were unable to provide appropriate advice to a pregnant woman complaining of severe headaches in the last trimester of her pregnancy. This suggests that Jordanian pharmacists may lack the necessary skills to optimize medication use during pregnancy.

Study Limitations

While this study proactively investigated the preparedness of Jordanian pharmacists to provide appropriate pharmaceutical care for pregnant women, it has several limitations that should be highlighted. First, the study had a relatively small sample size. Second, we recruited a random sample of pharmacists practicing exclusively in Amman, the capital of Jordan. Therefore, we cannot extrapolate our results to pharmacists practicing in other parts of the country. Third, there was an underrepresentation of male and older pharmacists with longer professional experience. This may be partly attributed to the high non-response rate to the

questionnaire. Additionally, it is possible that younger females were more interested in the study topic, which is related to women's health. Consequently, the generalizability of the results to the broader population of Jordanian pharmacists may be limited. Fourth, the web-based nature of the study, which utilized a self-administered questionnaire, is a potential weakness. This approach could introduce socially desirable responses and may not accurately reflect the pharmacists' actual knowledge, as some participants might have conducted a literature search before responding to the questions.

CONCLUSION

Pharmacists have demonstrated an inadequate level of preparedness in providing appropriate pharmaceutical care for pregnant women. Consequently, there is an urgent need for collaborative efforts between national health authorities and academic institutions to empower pharmacists and enhance their knowledge and skills necessary for optimizing the health outcomes of pregnant women.

The Jordan Pharmaceutical Association should develop a range of educational sessions aimed at providing Jordanian pharmacists with continuous and up-to-date knowledge about medications during pregnancy. Additionally, Jordanian universities should consider incorporating official courses into their curriculum that address pharmacotherapy during pregnancy.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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تقييم معايير العلاج الدوائي أثناء الحمل لدى خريجي كليات الصيدلة الأردنية

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ملخص

خلفية الدراسة: تعتبر معرفة الصيادلة بمخاطر الأدوية وفوائدها أثناء الحمل، وكفاءتهم في اتخاذ القرارات العلاجية المناسبة لتحسين استخدام الأدوية بين النساء الحوامل أمرًا محوريًا. تهدف هذه الدراسة إلى تقييم معرفة الصيادلة الأردنيين بمخاطر الأدوية وسلامتها أثناء الحمل، بالإضافة إلى تقييم قدراتهم على اتخاذ القرارات العلاجية المناسبة وتحسين استخدام الأدوية.

المنهجية: تم ارسال استبيان ذاتي يتكون من ستة أقسام مع خيارات محددة مسبقًا إلى 400 صيدلي تم اختيارهم عشوائياً في عمان، الأردن.

النتائج: أكمل 233 صيدلي الاستبانة بنسبة استجابة 58.2٪. ما يقرب 73.4 ٪ من الصيادلة حددوا الدواء الأفضل لارتفاع ضغط الدم أثناء الحمل. كما حدد أكثر من 70٪ من الصيادلة الجرعة الصحيحة من الأسبرين للوقاية من تسمم الحمل. وأظهر حوالي 50٪ من الصيادلة معرفة متدنية بمخاطر الأدوية وسلامتها أثناء الحمل.كما كان هناك فرق واضح في درجات الصيادلة في جميع الاختبارات بناءً على الحالة الاجتماعية و سنوات الخبرة سنوات. كما ذكر 79.8% من الصيادلة انهم درسوا المعالجة الدوائية اثناء الحمل في دراستهم الجامعية.

الاستنتاج: أظهر الصيادلة مستوى غير كافٍ من الاستعداد في تقديم الرعاية الصيدلانية المناسبة للحوامل. لذلك، هناك حاجة ملحة لدمج الجهود بين السلطات الصحية الوطنية والمؤسسات الأكاديمية لتمكين الصيادلة وتحسين معارفهم ومهاراتهم اللازمة لتحسين الرعاية الصحية للمرأة الحامل.

الكلمات الدالة: الحمل، العلاج الدوائي، السلامة الصحية، الرعاية الصيدلانية.

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Evaluation of Diuretic Property of *Argemone mexicana* along with Molecular Docking Study

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ABSTRACT

Background: Argemone mexicana L. (A. mexicana) has traditionally been used to treat hypertension, urinary issues, and constipation. In this study, we assessed the diuretic activity of the ethanolic crude extract of A. mexicana.

Methods: Phytochemical tests were conducted using standard reagents and methods widely accepted in the field. The diuretic test was performed in metabolic cages using a mouse model, with furosemide (5 mg/kg) as the standard drug. Molecular docking was carried out in PyRx using Autodock Vina 4.2. To assess the stability of the protein-ligand complexes formed during docking, we conducted molecular dynamics (MD) simulations for the β-amyrin-6PZT protein complex and the furosemide-6PZT protein complex. Various parameters, including RMSD, RMSF, Rg, SASA, and hydrogen bonds, were calculated for all protein-ligand complexes.

Results: Phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, steroids, terpenoids, saponins, and tannins in the crude extract. The crude extract exhibited significant ($p < 0.05$) diuretic activity compared to the control group. Furthermore, we detected the presence of electrolytes (Na+, K+, and Cl-) in the urine of mice treated with the crude extract. In the molecular docking study, among the eighteen compounds studied, β-amyrin displayed superior diuretic potential. The results of the molecular dynamics simulation for the β-amyrin-6PZT protein complex indicated good stability, comparable to the reference drug, furosemide.

Conclusion: The crude extract of A. mexicana demonstrates significant diuretic effects that could be valuable for edema treatment. The findings from the molecular docking and molecular dynamics simulations suggest the potential for further research in developing a novel drug.

Keywords: *Argemone mexicana*, Papaveraceae, Diuretic, Molecular docking, β-amyrin.

1. INTRODUCTION

Many plants are rich sources of various phytochemicals that can be utilized in designing and synthesizing medicines[1]. It is well-established that numerous medicinal plants possess potent diuretic properties, and they are commonly employed in conventional treatments for renal disorders[2-4]. Additionally, the diuretic properties of various plants used in ethnomedicine have been verified through animal studies[5].

Diuretics are medications that enhance the excretion of sodium ions (Na+) and urine production. They find application in the management of various clinical conditions, including hypertension, congestive heart failure, renal failure, and nephrotic syndrome, to regulate the volume and composition of body fluids. Diuretics can also influence the renal regulation of uric acid and the

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levels of various ions such as potassium (K+), hydrogen (H+), calcium (Ca2+), and magnesium (Mg2+), as well as anions like chloride (Cl−), bicarbonate (HCO3−), and dihydrogen phosphate (H2PO4−), in addition to altering Na+ excretion[6].

Diuretics, whether used alone or in combination with other medications, are employed in the treatment of conditions like congestive heart failure, ascites, and pulmonary edema[7-11]. Thiazides and furosemide, two commonly prescribed diuretics, have been associated with several side effects, including electrolyte imbalances, metabolic syndromes, and activation of the renin–angiotensin–neuroendocrine system[7, 10]. Consequently, there is a need for new diuretics with fewer adverse effects, such as those derived from plants, which are generally considered safer.

The Na-K-2Cl cotransporter-1 (NKCC1), also known as 6PZT, is an electroneutral Na+-dependent transporter responsible for the simultaneous transport of Na+, K+, and Cl− ions into cells[12, 13]. In human tissue, 6PZT plays a vital role in regulating cytoplasmic volume, fluid intake, chloride balance, and cell polarity[14-16]. Furosemide and other loop diuretics, frequently used to manage edema and hypertension, inhibit this transporter[17, 18]. Our computer-aided molecular modeling of the binding sites of loop diuretics at 6PZT was prompted by these findings.

A. mexicana L., also known as Ghamoya and a member of the Papaveraceae family, is a rare weed that originated in South America but is now common in many Asian nations, including China and India[19, 20]. The plant is an annual herb, reaching a height of approximately 1 meter; its leaves typically measure 5-11 cm in length and are spiny[21]. The flowers are 4-5 cm in diameter and yellow[22]. Various parts of this plant have been reported to be used in the treatment of conditions such as hypertension, oliguria, diarrhea, ulcers, asthma, and other intestinal afflictions[21, 23-30].

Furthermore, researchers have identified various bioactive compounds in the plant, including alkaloids such as berberine, (+)-reticuline, allocryptopine, (-)-cheilanthifoline, (+)-argenaxine, (+)-higenamine, and N-demethyloxysanguinarine[31, 32], flavonoids, e.g., eriodictyol, isorhamnetin-3-*O*-β-Dglucopyanoside, quercetin, quercetrin, mexitin [33-36], terpenoids, e.g., *trans*-phytol, β-amyrin [32, 34] steroids, e.g., β-sitosterol [37]; miscellaneous, e.g., α-tocopherol, adenosine, adenine, myristic acid, oleic acid, linoleic acid [32, 38], amino acids, e.g., cysteine, phenylalanine[34].

Upon literature survey, based on ethnopharmacological uses of *A. mexicana* in hypertension, oliguria [24], the entire plant was selected for evaluating its diuretic properties in a mouse model. This was followed by a molecular docking and molecular dynamics simulation analysis of the previously reported compounds.

2. MATERIALS AND METHODS

2.1 Collection and identification of *A. mexicana*

The whole plant of *A. mexicana* was collected for this investigation from the Chuknagar area of Jashore, Bangladesh, in January 2017. A voucher specimen of *A. mexicana* was submitted to the Bangladesh National Herbarium in Mirpur, Dhaka (DACB Accession number: 43825) for future reference.

2.2 Drying, grinding, and Cold extraction

The collected whole plants were cleaned and carefully inspected to remove any unwanted objects, other plants, or weeds. They were air-dried for a week. Subsequently, the plant material was ground into a coarse powder using a suitable grinder. The resulting powder was stored in an airtight container, maintaining a cool, dark, and dry environment until the extraction process.

Approximately 800 g of the powdered material was placed in a clean, flat-bottomed glass container and dissolved in 1500 mL of ethanol. The glass container was covered with aluminum foil, and the mixture was allowed to sit for 14 days with intermittent stirring. Afterward, the contents of the glass container were filtered, first through a piece of cotton and then through Whatman® filter paper. The resulting filtrate was subjected to evaporation using a

rotary evaporator, yielding a sticky dark gummy crude extract weighing 18.12 g, with a yield of 2.26%.

2.3 Phytochemical screening

Phytochemical testing was conducted to identify chemical groups present in the test extracts. Various phytochemical experiments on the crude extract were performed using standard techniques[39-43].

2.4 Experimental animal

Swiss-albino mice were sourced from Jahangirnagar University in Savar, Bangladesh, and then raised for one week under standard conditions in the animal lab of the Pharmaceutical Department at Khulna University, Bangladesh. These animals were kept in a natural lighting environment and provided with standard laboratory food and water. The study employed young Swiss albino mice, aged 4-5 weeks, with an average weight of 25-30 g. All procedures adhered to the animal ethical standards established by the Life Science School at Khulna University, Bangladesh (Reference: KUAEC-2018/05/10).

2.5 Acute toxicity study

The acute toxicity assessment was conducted in mice following the recommendations of the Organization for Economic Co-operation and Development (OECD)[44-46]. The crude extract was administered to three groups of mice at doses of 1000, 2000, and 3000 mg/kg, and differences in mortality and body weight were recorded in comparison to a control group. Individual post-dose observations were made during the first 30 minutes, periodically over the first 24 hours, and daily over the subsequent 14 days.

2.6 Diuretic activity evaluation

The diuretic test procedure followed a pre-established methodology adapted by Golla and colleagues with minor modifications[47-50].

In brief, twenty-four mice of both sexes, weighing 27-30 g, were divided into four groups of six each. Each group underwent an 18-hour fasting period without access to food or water before the test. The first group received normal saline (6x2 mL) as a control. The second group (standard) received furosemide (5 mg/kg) as a positive control. The third and fourth groups were administered the crude extract at doses of 200 and 400 mg/kg, respectively. Each group received a total volume of 6 × 2 mL (Vi). Following dosing, the animals were placed in metabolic cages. Throughout the six-hour experiment, the mice were deprived of food and water, and the cages were maintained at a constant temperature of 25.0±0.5 °C. Urinary output (V0) was measured hourly, and the urine was stored at 0–4 °C for later electrolyte measurement. The urinary excretion was estimated using the ratio of urine output (V0) to the total liquid delivered (Vi) (Formula-I). The diuretic action was calculated as the ratio of urinary excretion in the test group (UET) to that in the control group (UEC) (Formula-II). Diuretic activity was determined by the ratio between the diuretic action in the test group (DAT) and that in the control group (Formula-III).

$$\text{Urinary excretion} = \frac{\text{Total urinary output } (V_0)}{\text{Total liquid administered } (V_i)} \times 100 \ldots \text{(I)}$$

$$\text{Diuretic action} = \frac{\text{Urinary excretion of treatment groups}}{\text{Urinary excretion of the control group}} \ldots\ldots\ldots \text{(II)}$$

$$\text{Diuretic activity} = \frac{\text{Diuretic action of test groups}}{\text{Diuretic action of standard group}} \ldots\ldots\ldots \text{(III)}$$

2.7 Analysis of Urine Sample for different cations and anions

The D-50 Series Portable Water Quality Meters from HORIBA Scientific® were employed to measure the pH and conductivity of the preserved urine samples. The concentrations of Na+ and K+ in the samples were determined using a flame photometer, specifically the Janeway Corp. model PFP7. Flame intensity was measured for Na+ and K+ concentrations with appropriate filters, aligning with calibration standards. Results were displayed graphically, and Na+ and K+ concentrations were calculated from the standard curve and expressed in mEq/L[51]. The Cl− content was determined through titration with a 0.05 N silver nitrate solution, utilizing 5% potassium chromate solution as an indicator[52, 53]. To predict the mechanism of action of diuretic drugs, several

indices were calculated for the preserved urine samples, including the saluretic index, natriuretic index (Na+/K+ ratio), kaliuretic index (K+/Na+ ratio), and carbonic anhydrase inhibition (CAI) index.

Here,

$$\text{Saluretic index} = \frac{\text{Urinary excretion of electrolytes in the test group}}{\text{Urinary excretion of electrolytes in the control group}}$$

$$\text{Natriuretic Index} = \frac{\text{Urinary excretion of Sodium ion}}{\text{Urinary excretion of Potassium ion}}$$

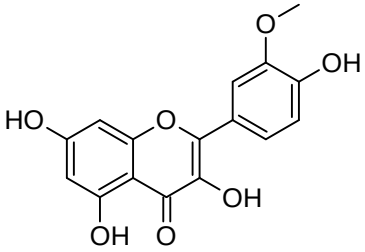
$$\text{Kaliuretic index} = \frac{\text{Urinary excretion of Potassium ion}}{\text{Urinary excretion of Sodium ion}}$$

$$\text{CAI index} = \frac{\text{Urinary excretion of Chloride ion}}{\text{Sum of Urinary excretion of Sodium and Potassium ions}}$$

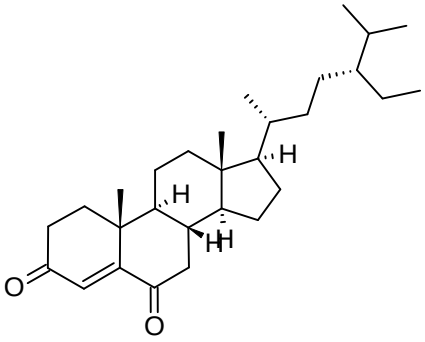
2.8 Molecular docking analysis

Preparation of the ligands

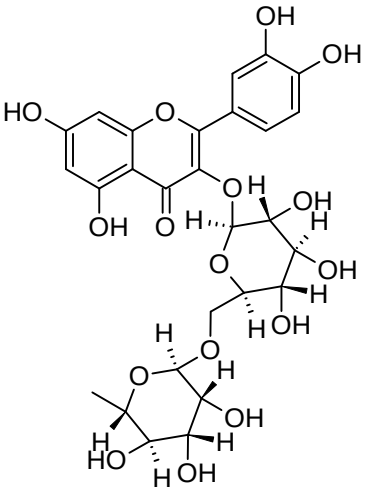
3D structures of already reported eighteen compounds (Figure 1) [22] were retrieved from PubChem (https://pubchem.ncbi.nlm.nih.gov/). PyRx was then used for energy minimization [44].



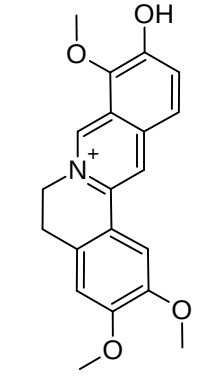
isorhamnetin



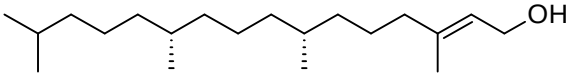
stigmast-4-ene-3,6-dione



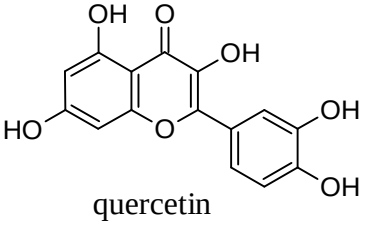
rutin



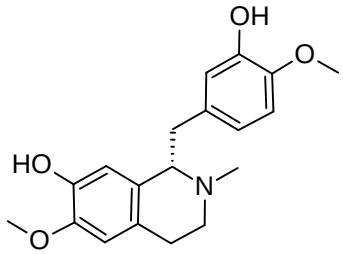
dehydrocorydalmine



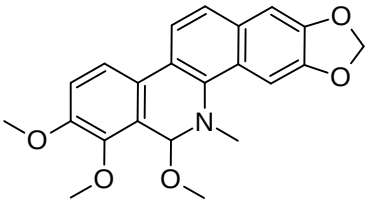
phytol



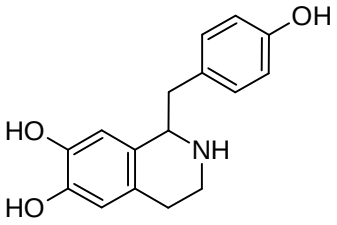
quercetin



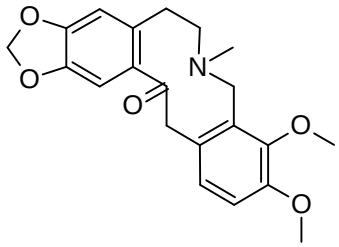
reticuline



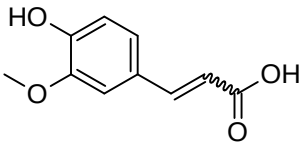
angoline



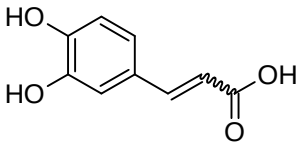
higenamine



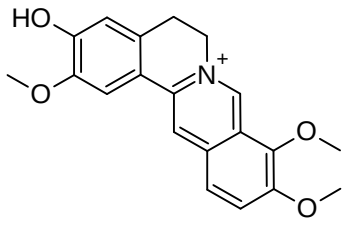
allocryptopine



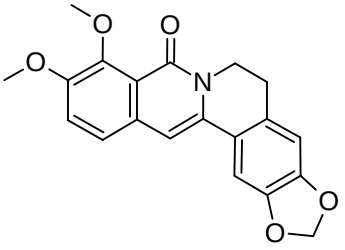
ferulic acid



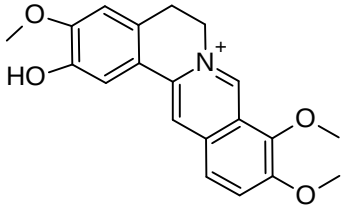
caffeic acid



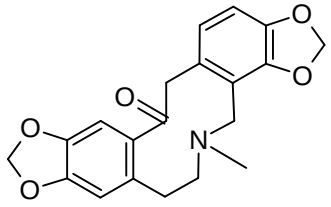
jatrorrhizine



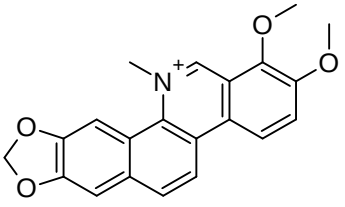
oxyberberine



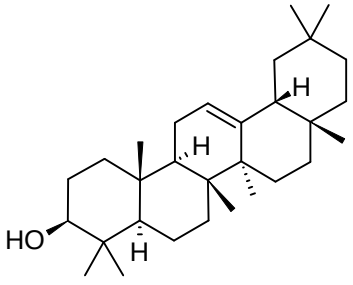
columbamine



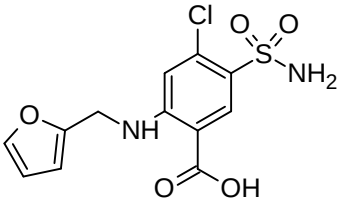
protopine



chelerythrine



β-amyrin



furosemide

Figure 1: Structure of eighteen compounds reported from A. *mexicana* and furosemide.

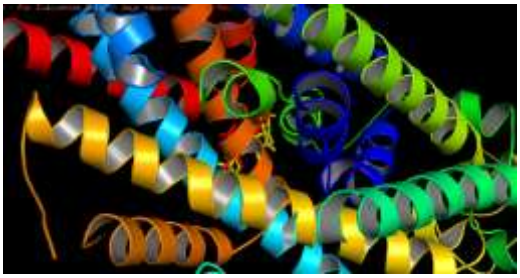


Figure 2: Binding region of docked furosemide (red) and β-amyrin (yellow) with 6PZT protein.

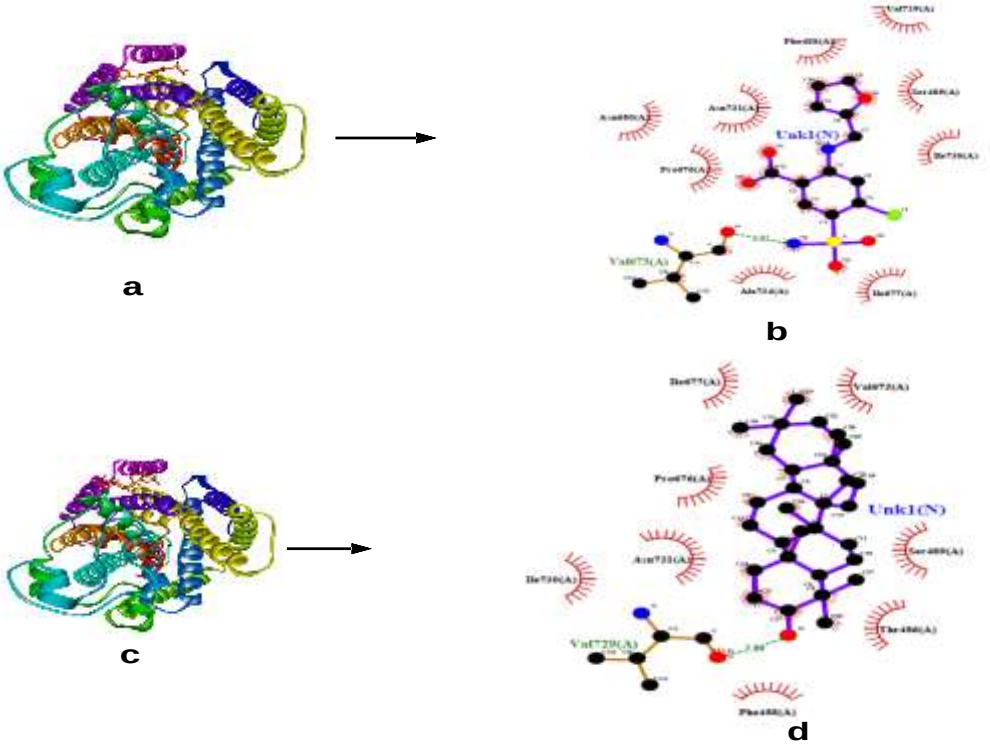


Figure 3: (a) Furosemide and (c) β-amyrin protein complex with 6PZT; (b) furosemide and (d) β-amyrin 2D interaction.

β-amyrin-6PZT protein complex

furosemide -6PZT complex

apo-protein

Preparation of the protein

The 6PZT protein was obtained from the Protein Data Bank (https://www.rcsb.org). Using Discovery Studio Visualizer, the retrieved protein underwent cleaning, and

polar hydrogen atoms were added. Since 6PZT forms a dimer with two-fold symmetry, we considered a single chain for docking[54]. Additionally, SwissPDB Viewer was utilized for energy minimization[55].

Molecular docking and visualization

Amino acid residues were selected for docking using AutoDock Vina 4.2 in PyRx[56]. The grid box was maximized to cover the entire protein. LigPlot Plus 2.2.4 was employed to analyze the outcomes. Through molecular docking, we identified interactive amino acids with the target proteins and determined the binding affinities of the selected drugs.

Molecular dynamics simulations

GROMACS 2021 was utilized in simulations of molecular dynamics (MD) using the charmm36 force field[57, 58]. The ligands parameters were generated using the CGenFF server for CHARMM General (cgnff.umaryland.edu)[59]. A decahedron box with a TIP3P water model was used to dissolve protein-ligand complexes. The system was neutralized by the use of Na^+ and Cl^- ions. Following minimization, the system was run with coupled temperature and pressure control using the NVT and NPT ensemble at 310 K and 1 bar. The final MD run lasted 100 nanoseconds. To assess relative stability, root mean square deviation (RMSD), root mean square fluctuations (RMSF), radius of gyration (Rg), solvent-accessible surface area (SASA), and hydrogen bond analyses were performed.

2.9 Statistical Analysis

Data were analyzed using Student's unpaired t-test in GraphPad Prism Version 5.03, and the results were presented as Mean ± Standard Error of Mean (S.E.M) (GraphPad Software, San Diego, CA, USA). All in vivo study outcomes were compared with those of the control group, and a significance level of $P < 0.05$ was applied.

3. RESULT

3.1 Preliminary phytochemical study

This study consisted of testing several chemical groups found in the extract. The outcome of the phytochemical study of the crude extract is summarized in **Table 1**.

Table 1: Results of phytochemical study of *A. mexicana* crude extract

Phytochemical groups	Results
Reducing sugars	−
Tannins	+
Flavonoids	+
Saponins	+
Steroids	+
Alkaloids	+
Glycosides	+
Terpenoids	+
Acidic compounds	+

Here, + indicates Presence; − indicates Absence

3.2 Acute toxicity study

This investigation provided evidence of the extract's non-toxicity. None of the doses used resulted in fatalities or adverse responses until the end of the trial period, suggesting that the LD50 of the extract may exceed 3000 mg/kg.

3.3 Diuretic activity test

The different parameters for assessing diuretic activity

in the extract, control, and standard groups are presented in Tables 2, 3, and 4. Table 2 displays information on urine volume, urinary excretion, diuretic action, and diuretic activity. Table 3 presents data on the diuretic index, pH, and conductivity, while Table 4 provides information on the electrolyte concentrations (Na+, K+, Cl−) in mEq/L, saluretic index, Na+/K+ ratio, and CAI in the urine of animals treated with the extract, furosemide, and untreated control groups.

Table 2: Effects of *A. mexicana* crude extract on volume of urine, urinary excretion, diuretic action and diuretic activity in mice

Group	Cumulative urine volume (V_0 mL) after 6h	Urinary excretion {(V_0/V_i) X 100}	Diuretic action (U_{ET}/U_{EC})	Diuretic activity (D_{AT}/D_{AF})
Control (Normal Saline)	3.35±0.15	27.91	1.00	–
Furosemide (5 mg/kg)	5.1±0.10[b]	42.50	1.52	1.00
Crude Extract (200 mg/kg)	3.85±0.25	32.08	1.14	0.75
Crude Extract (400 mg/kg)	4.9±0.80[a]	40.83	1.46	0.96

Values are expressed as Mean ± S.E.M; n = 6; [a]$P < 0.05$, [b]$P < 0.001$, compared to the control group

Table 3: Effects of *A. mexicana* crude extract and furosemide on urinary volume, diuretic index, conductivity and pH in mice

Group	Diuretic Index	pH	Conductivity (mS/cm)
Control (Normal Saline)	1	7.14 ± 0.02	6.05 ± 0.45
Furosemide (5 mg/kg)	1.52	7.36 ± 0.04[b]	16.77 ± 0.02[c]
Crude extract (200 mg/kg)	1.14	7.05 ± 0.03	15.74 ± 0.01[b]
Crude extract (400 mg/kg)	1.46	7.79 ± 0.04[b]	11.59 ± 0.05[b]

Values are expressed as Mean±S.E.M; n = 6; Diuretic index = Urine volume of test group/ Urine volume of control group; [a]$P < 0.05$. [b]$P < 0.01$, [c]$P < 0.001$, compared to the control group (Student's unpaired *t*-test).

Table 4: Effect of the crude extract and furosemide on urinary electrolytes excretion in mice

Group	Cumulative Concentrations of ions (mEq/L/6h)			Saluretic Index			Na^+ / K^+	K^+/ Na^+	CAI {Cl^-/ (Na^+ + K^+)}
	Na^+	K^+	Cl^-	Na^+	K^+	Cl^-			
Control (Normal Saline)	49.5±6.13	29.5±0.74	63.3±3.63	–	–	–	1.67	0.59	0.80
Furosemide (5 mg/kg)	134.4±6.13[c]	53.4±1.47[c]	78.3±2.20[b]	2.71	1.81	1.23	2.51	0.39	0.41
Crude Extract (200 mg/kg)	187.5±6.13[b]	36.3±1.54[a]	91.7±2.20[a]	3.79	1.23	1.44	5.17	0.19	0.40
Crude Extract (400 mg/kg)	215.8±9.36[b]	73.2±1.29[b]	95.0±1.44[b]	4.36	2.48	1.50	2.94	0.33	0.32

Values are expressed as Mean ± S.E.M; n = 6; [a]$P < 0.05$, [b]$P < 0.01$; [c]$P < 0.001$; compared with the control group (Student's unpaired *t*-test).

3.4 Effect on urine volume, pH, and conductivity

The urine volume for both doses (200 and 400 mg/kg) of the crude extract (3.85 ml, 4.90 ml) was found to be higher compared to the control (3.35 ml) (Table 2).

The pH of the urine increased dose-dependently at both doses for the crude extract (7.05, 7.79) compared to the control (7.14) (Table 3). Additionally, specific conductivity was enhanced by the crude extract (15.74, 11.59) compared with the control (6.05) (Table 3).

3.5 Effects on electrolyte excretion

Our findings demonstrated that the excretion of Na+ (measured in mEq/L) from the crude extracts increased at both 200 and 400 mg/kg (187.5, 215.8). Furthermore, an increase in the excretion of potassium ions was observed for the crude extracts (36.3, 73.2) at both 200 and 400 mg/kg. Additionally, an elevation in the excretion of chloride ions at both 200 and 400 mg/kg for the crude extracts (91.7, 95.00) was found (Table 4). The saluretic index, natriuretic index, kaliuretic index, and CAI index increased dose-dependently for the crude extract (Table 4).

3.6 Molecular docking analysis

β-Amyrin displayed a superior binding affinity (-8 kcal/mol) with the protein compared to the standard furosemide (-5.8 kcal/mol). Additionally, other compounds such as protopine, both chelerythrine and Stigmast-4-ene-3,6-dione, rutin, and angoline also exhibited better binding affinities of 7.7, 7.3, 7.2, and 7.1 kcal/mol, respectively (Table 5).

Table 5: Docking results of the selected reported ligand compounds with 6PZT protein

Compounds Name	Binding Affinity (kcal/mol)	Interacting amino acids
Isorhamnetin	−6.6	Phe488, Val729, Ala492, Ile730, Pro676, Ser489, Asn731, Asn672, Ala734
Stigmast-4-ene-3,6-dione	**−7.3**	Ile324, Leu531, Thr530, Met325, Val534, Val321, Thr328, Leu527, Ile526, Thr332, Leu523
Rutin	**−7.2**	Ile677, Asn731, Val673, Val729, Pro676, Ile730, Thr486, Ile493, Asn672, Ser489
Dehydrocorydalmine	−6.5	Ile677, Val673, Asn731, Ile730, Pro676, Phe488, Ala492, Thr486, Ser489, Val729, Glu484
Phytol	−5.4	Phe488, Val729, Ile730, Ala492, Ser489, Pro676, Asn731, Asn672, Ala734, Trp733,Val673,Ile677, Phe681, Asn680
Quercetin	−6.8	Glu484, Ile493, Asn672, Glu485, Pro676, Asn680, Ile730, Asn731, Ile677, Ala734
Reticuline	−6.3	Ser489, Ile493, Asn672, Glu485, Pro676, Asn731, Ile677, Ala734, Asn680, Phe681
Angoline	**−7.1**	Ser489, Asn731, Pro676, Ile730, Val673, Ala734, Trp733, Ile677, Asn680
Higenamine	−6.4	Asn672, Val673, Asn731, Glu485, Pro676, Ser489, Ile730, Phe488, Thr486
Allocryptopine	**−7**	Ile677, Asn731, Pro676, Ile730, Thr486, Phe488, Ser489, Val729
Ferulic acid	−5.1	Phe488, Ser489, Asn731, Ile730, Pro676, Ile677, Asn680, Ala734, Val673
Caffeic acid	−5.1	Ala434, Leu612, Ser613, Leu438, Gln435, Leu297, Arg294
Jatrorrhizine	−6.7	Gly664, Phe665, Trp733, Ile677, Asn731, Pro676, Ala734, Asn680
Oxyberberine	−6.9	Ile677, Ile668, Trp733, Val673, Asn731, Pro676, Ser489
Columbamine	−6.1	Leu523, Leu527, Thr332, Thr328, Val321, Val534, Thr530
Protopine	**−7.7**	Ile677, Pro676, Asn731, Phe488, Ile730, Ser489, Val729, Thr486
Chelerythrine	**−7.3**	Ala734, Ile677, Asn680, Asn731, Pro676, Ala492, Ile730
β-Amyrin	**−8**	Ile677, Val673, Pro676, Asn731, Ser489, Ile730, Thr486, Val729, Phe488
Furosemide	−5.8	Val729, Phe488, Ser489, Asn731, Asn680, Ile730, Pro676, Val673, Ala734, Ile677

Compounds marked bold indicated the better binding affinities.

3.7 Molecular dynamics simulation

RMSD considers deviations between two three-dimensional structures over time[60]. Over the course of 100 ns, we analyzed the RMSD of backbone atoms in the apo-protein, furosemide-protein complex, and β-amyrin-protein complex to assess the stability of all systems (Figure 4a).

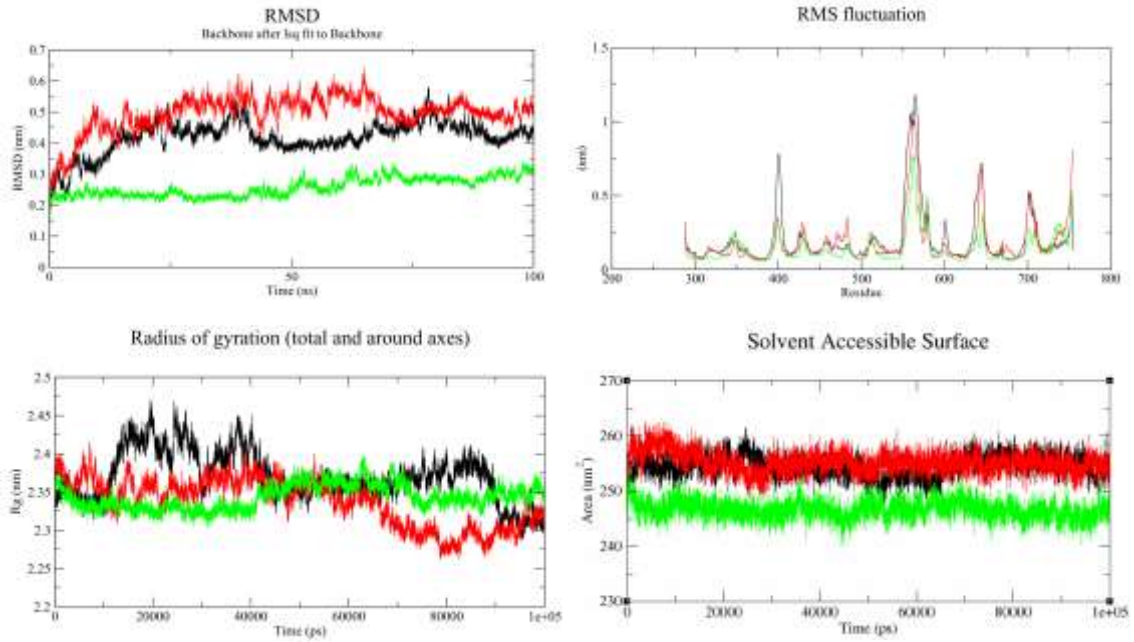


Figure 4: a) Plot of RMSD of backbone atoms vs. time (in nano seconds), b) RMSF of backbone atoms versus residue number, c) Radius of gyration (Rg) versus time (in pico seconds), d) solvent accessible surface area (SASA) versus time (in pico seconds) for β-amyrin-6PZT protein complex (black), furosemide -6PZT complex (red), and for apo-protein (green)

In the initial 20 ns, structural rearrangements were followed by minor conformational alterations in all systems. Apoprotein underwent multiple conformational changes throughout the investigation. The RMSD value of the β-amyrin-protein complex fluctuated between 0.0005 and 0.58 nm, with an average value of 0.42 nm and no significant spikes, indicating that β-amyrin effectively stabilized the protein by binding to it. In contrast, the furosemide-protein complex exhibited a steadily increasing trend in RMSD.

Regarding the overall RMSD for all systems, the apo-protein fluctuated within the range of 0.006-0.34 nm, with an average RMSD of 0.26 nm. On the other hand, the RMSD of the β-amyrin-protein complex and the furosemide-protein complex ranged from 0.0005 nm up to 0.42 nm and 0.50 nm, respectively. Our comprehensive RMSD analysis suggests that β-amyrin is more effective than furosemide in stabilizing the protein.

RMSF is the fluctuations observed in residues or atoms present in a macromolecule[61]. In this study, we assessed the RMSF of backbone residues in both the apo-protein and all protein-ligand complexes (Figure 4b). Even in regions experiencing the most significant changes, the RMSF graphs exhibited a similar profile, with average RMSF values of 0.21 nm for the β-amyrin-protein complex and 0.18 nm for the furosemide-protein complex.

However, our RMSF analysis indicated that the β-amyrin-protein complex stabilizes the protein in a manner similar to that of the furosemide-protein complex.

Rg is a routinely used parameter to predict the compactness of macromolecules[62]. In this study, we analyzed the Rg of the apo-protein and all protein-ligand complexes (Figure 4c). The β-amyrin-protein complex exhibited a higher Rg until 40 ns, after which it showed a lower Rg, indicating stability. However, the average Rg value of the β-amyrin-protein complex was 2.36 nm, which was comparable to that of the furosemide-protein complex (average Rg value = 2.34 nm).

A molecule's surface area that interacts with the solvent molecules is its solvent-accessible surface area [63]. The β-amyrin-protein complex and the furosemide-protein complex had average SASA values of 254.54 nm^2 and 255.21 nm^2, respectively (Figure 4d). Based on the SASA values for all complexes, it appeared that the furosemide-protein complex was more exposed to water solvent than the β-amyrin-protein complex. Our results indicate that the protein coupled with β-amyrin was highly stable and compact.

Number of hydrogen bonds

Hydrogen bond interactions play a crucial role in stabilizing macromolecules and are directly related to binding affinity and drug efficacy[64, 65]. Figure 5 illustrates the number of hydrogen bonds formed during the 100 ns simulation run as a result of interactions between the protein and ligand combinations. Based on our hydrogen bond observations, all compounds maintained optimized hydrogen bonding for up to 25 ns. This suggests that the chemical can have the necessary impact on drug specificity, metabolism, and adsorption.

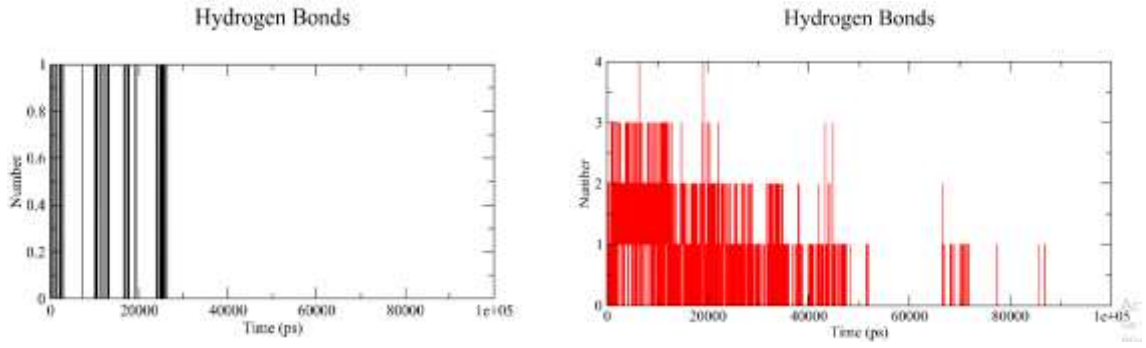


Figure 5: Plot of Number of hydrogen bonds versus time (in picoseconds) for β-amyrin-6PZT protein complex (black), furosemide -6PZT protein complex (red)

4. DISCUSSION

The phytochemical tests conducted on the extract revealed the presence of several important constituents, including tannins, flavonoids, glycosides, saponins, steroids, etc. Previous studies have shown that these types of phytochemicals are responsible for diuretic activity [66, 67]. Therefore, these compounds may be responsible for the traditional medicinal uses associated with diuretic activity. Consequently, further studies targeting the assessment of the diuretic potential of the plant were conducted.

The current study aimed to assess the diuretic properties of the extract. Two key indicators of diuresis are an increase in net urinary volume and elevated excretion of electrolytes in the urine[9]. These processes result from the inhibition of water and electrolyte reabsorption into the circulation in the renal tubules. Thiazide diuretics restrict the Na+/Cl− symporter (co-transporter system) in the distal tubule by competing for the Cl− binding site, while the standard loop diuretic drug, furosemide, increases urinary Na+ excretion by inhibiting the Na+/K+/Cl−

symporter in the thick ascending limb of the loop of Henle[9]. Therefore, in this study, both urine volume and electrolyte concentrations were evaluated to assess the diuretic effect of the A. mexicana crude extract. In the current investigation, a dose of 5 mg/kg of furosemide produced significant diuresis in mice for 6 hours. Our findings indicate that the extract increased urine volume and urinary excretion (Table 2). We observed a notable and significant increase in the excretion of Na+, K+, and Cl− compared to the control group, similar to what was observed with furosemide (Table 3). Additionally, the extract exhibited a progressive increase in electrolyte excretion (Na+, K+, and Cl−) in a dose-dependent manner (Table 4).

A saluretic activity metric was defined as the amount of Na+, K+, and Cl− excretion[68]. The extract exhibited dose-dependent electrolyte and water excretion to varying degrees. To assess natriuretic activity, the ratio of Na+ to K+ was calculated[69, 70]. Values greater than 2.0 indicate a beneficial natriuretic effect, while ratios greater than 10.0 suggest a potassium-sparing effect[71]. The Na+/K+ values (Table 4) for the crude extract at their respective doses of 200 and 400 mg/kg were 2.94 and 5.17, respectively. This result falls within the acceptable value range of 2, suggesting that the extract possesses a potent natriuretic effect.

Previous studies have reported that several bioactive compounds contribute to the diuretic effect, including flavonoids, saponins, triterpenoids[70, 72-74] glycosides [66, 70, 75], tannins [70, 72] and steroids [67]. These phytochemicals may induce the diuretic effect by stimulating regional blood flow, initial vasodilation, inhibiting water and electrolyte reabsorption by tubules, or enhancing renal circulation, ultimately leading to diuresis[76]. The preliminary phytochemical study of the extract confirmed the presence of the aforementioned phytochemicals. Therefore, it can be inferred that these phytochemicals, alone or in combination, may be responsible for the diuretic activity of the plant. Additionally, compounds such as quercetin[77], caffeic acid [78], and rutin [79] present in the plant, have been reported to exhibit diuretic activities. These compounds could contribute to the diuretic properties of the A. mexicana crude extract.

The human cation–chloride cotransporter NKCC1 is the target protein for the standard drug Furosemide. Inhibiting this cotransporter is the mechanism of action for loop diuretics[54]. Therefore, the activity of the test ligands was assessed against this cotransporter protein and compared with the standard, Furosemide. Among the previously reported compounds from this plant, β-Amyrin, a triterpene alcohol, displayed a binding affinity of −8 kcal/mol, which is almost 1.4 times greater than that of Furosemide (−5.8 kcal/mol) (Table 5), and the binding site of these two compounds is identical, as shown in Figure 2. While the standard drug Furosemide interacted with amino acids Phe488, Ser489, Val673, Pro676, Ile677, Asn680, Val729, Ile730, Asn731, and Ala734, β-amyrin interacted with amino acids Thr386, Phe488, Ser489, Val673, Pro676, Ile677, Val729, Ile730, and Asn731. Amino acids Phe488, Ser489, Val673, Pro676, Ile677, Val729, Ile730, and Asn731 are common to both compounds (Figure 3). Furosemide formed a hydrogen bond with the Val673 amino acid, and β-amyrin formed a hydrogen bond with Val729. Hydrogen bonding is crucial for protein stabilization as it maintains specific shapes. Here, the presence of the hydrogen bond indicates the stable protein complex of β-amyrin with 6PZT. Other amino acids, such as Phe488, Ser489, Pro676, Ile677, Ile730, and Asn731, all formed hydrophobic interactions with the protein. Therefore, β-amyrin could also play a role in diuretic activity. Our literature findings also revealed that terpenoid-enriched plant extracts promote diuresis, which supports this result[74].

5. CONCLUSION

The results of in vivo studies on diuretic activity support the ethnopharmacological use of A. mexicana for treating urinary problems. In the molecular docking study, among the eighteen reported compounds, β-amyrin, a

triterpene alcohol, exhibited superior diuretic potential. RMSD, RMSF, Rg, hydrogen bonds, and energy analysis all demonstrated the stable binding of the β-amyrin-6PZT protein complex. This stable binding supports the diuretic activity of Argemone mexicana. Therefore, this evidence can be utilized for discovering the mechanism of action and investigating the pharmacodynamic and pharmacokinetic parameters.

Declaration of interest

None.

Consent for publication

All co-authors have consented to the publication of this manuscript.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the article.

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تقييم خاصية مدر للبول من Argemone mexicana مع دراسة الالتحام الجزيئي

بابيترا كومار سردار[1]، هيرون سراج ديفناث[1]، نريبندرا ناث بيسواس[1*]، بيشواجيت بوكشي[1*]، سمير كومار سادو[1]

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ملخص

الخلفية: تم استخدام Argemone mexicana L. (A. mexicana) تقليديًا لعلاج ارتفاع ضغط الدم ومشاكل المسالك البولية والإمساك. هنا، تم تقييم مستخلص الخام الإيثانولي A. mexicana للنشاط المدر للبول.

الطريقة: تم إجراء الاختبارات الكيميائية النباتية باستخدام الكواشف والطرق القياسية المقبولة عمومًا. تم إجراء اختبار مدر للبول في أقفاص أيضية باستخدام نموذج الفئران حيث تم أخذ فوروسيميد (5 مجم / كجم) كدواء قياسي. تم إجراء عملية الالتحام الجزيئي في PyRx باستخدام autodock vina 4.2. لتقييم ثبات مجمع بروتين–يجند الراسي، تم إجراء محاكاة الديناميكيات الجزيئية (MD) لمركب البروتين β-amyrin-6PZT ومركب بروتين فوروسيميد –6 PZT. تم حساب قيم RMSD و RMSF و Rg و SASA والروابط الهيدروجينية لجميع مجمعات البروتين – الترابط.

النتائج: أظهر الفحص الكيميائي النباتي وجود قلويدات، فلافونويد، جليكوسيدات، منشطات، تربينويد، صابونين، وتانينات. أظهر المستخلص الخام نشاطا مدر للبول معنويا ($P < 0.05$) مقارنة مع المجموعة الضابطة. علاوة على ذلك، تم الكشف عن وجود إلكتروليتات+ Na) ، + K، و Cl) في بول الفئران المعالجة بالمستخلص الخام. في دراسة الالتحام الجزيئي، من بين المركبات الثمانية عشر المبلغ عنها، وجد β-amyrin قدرة فائقة على إدرار البول. أظهرت نتيجة محاكاة الديناميكيات الجزيئية لمركب البروتين β-amyrin-6PZT استقرارًا جيدًا مقارنة بالفوروسيميد المرجعي.

الخلاصة: المستخلص الخام من A. mexicana له تأثيرات مدرة للبول يمكن استخدامها لعلاج الوذمة. قد تساعد نتيجة الالتحام الجزيئي ومحاكاة الديناميكيات الجزيئية في إجراء مزيد من الدراسات لتطوير دواء جديد.

الكلمات الدالة: Argemone mexicana، Papaveraceae، مدر للبول، الالتحام الجزيئي، β-amyrin.

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Echinomycin: A Journey of Challenges

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ABSTRACT

Echinomycin is a natural compound discovered and isolated from bacteria, introduced as a promising antibiotic and anticancer therapy. However, it failed clinically due to improper formulations and a short half-life. After the unsuccessful clinical trials, echinomycin was overlooked. Recently, a new mechanism of action has given some hope for reviving echinomycin as an inhibitor of hypoxia-inducible factor (HIF-1). In 2015, echinomycin received orphan drug designation for treating acute myeloid leukemia in the USA. Furthermore, advancements in drug delivery systems have provided new prospects to overcome the echinomycin formulation issues and explore further therapeutic benefits. This review details the echinomycin journey along with the main challenges of this potent drug and provides insights into possible future clinical applications.

Keywords: Echinomycin, targeted ligands, cyclic peptides, quinoxaline antibiotic, DNA bis-intercalator.

1. INTRODUCTION

Echinomycin (NSC526417) is a quinoxaline antibiotic peptide with a unique thioacetal bridge (2). It was initially isolated from Streptomyces echinatus bacteria in the 1950s and introduced as an antibiotic (3). It possesses potent antibacterial, anticancer, and antiviral activities. Echinomycin binds to double-strand DNA and intercalates into DNA at two specific sites, causing inhibition of DNA replication and RNA synthesis (4, 5). Echinomycin showed promise as a cytotoxic drug, leading to its progression to phase I and II clinical trials for various types of cancers (6). Research by Park et al. revealed that echinomycin is more effective against Staphylococcus aureus than vancomycin, both in vitro and in vivo in a mouse model. However, a major challenge in using echinomycin is its hydrophobic nature and water insolubility. Currently, echinomycin is under investigation for its antineoplastic effect as an inhibitor of hypoxia-inducible factor-1 (HIF-1), a critical factor in leukemia cell growth (7).

Furthermore, echinomycin has been observed to down-regulate numerous signaling pathways, including the Notch signaling pathway (3, 8). It is important to note that the most commonly reported toxicity associated with echinomycin is severe nausea and vomiting, a side effect that is comparable to other chemotherapeutic agents like actinomycin. To harness the full potential of this powerful drug, it is crucial to mitigate its toxicity and enhance its bioavailability and solubility (9). The objective of this current review is to comprehensively examine past and present research on echinomycin, shedding light on its potential future applications in clinical settings (10).

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2. ECHINOMYCIN DISCOVERY

Echinomycin was first discovered and isolated from Streptomyces echinatus species in Germany in 1957. Four years later, the same compound was produced by Streptomyces species in Japan and was given the name Quinomycin (11). Subsequently, more than thirty-seven members of the quinoxaline antibiotics were discovered (12). The identity of echinomycin (levomycin and quinomycin A) was conclusively determined in 1964. Quinomycin A was found to be identical to echinomycin based on paper chromatogram analysis of the compounds isolated from Streptomyces species (13).

A few years later, the mechanism of action of quinoxaline antibiotics was elucidated, revealing their interaction with deoxyribonucleic acid (DNA) (14). Waring and Wakelin described echinomycin's bifunctional intercalation activity with DNA (14). In 1975, the structure of echinomycin was reexamined and recharacterized using proton and carbon-13 nuclear magnetic resonance (NMR), electron impact, and field desorption mass spectrometry (15). Furthermore, through footprinting methods, a specific DNA binding site for echinomycin was identified as a 4-base pair sequence with the central two-base pair of 5'-CG-3' (16, 17).

Adams and Rinaldi conducted research on the effect of echinomycin on DNA methylation. They found that echinomycin does not inhibit DNA methylation, suggesting that methylation does not involve the transient separation of double strands. Instead, the primary effect of echinomycin was the inhibition of DNA and RNA synthesis (18).

Over the years, additional research and studies have been undertaken to further understand the potential of this highly potent drug. Echinomycin has been investigated for its antineoplastic effects, and its complete story, including its activity as an inhibitor of hypoxia-inducible factor 1 (HIF-1), has been newly developed (8, 19-21).

3. ECHINOMYCIN PROPERTIES

3.1. Physicochemical properties

Echinomycin is a hydrophobic, colorless, needle-like crystalline compound that is soluble in chloroform, dichloromethane, and dioxane but insoluble in water and hexane. Its distinction from other compounds was achieved using paper chromatography, where its retention factor (Rf) was determined to be 0.15 (22). In the early 1990s, the molecular model of echinomycin was defined through crystallographic data. Most color reactions with this compound are negative, except for the ninhydrin reaction in HCl at 100 °C. Echinomycin can be quantified in human plasma using High-Performance Liquid Chromatography (HPLC) (23, 24).

3.2. Echinomycin structure elucidation and biosynthesis

Echinomycin (NSC52641), also known as quinomycin A and levomycin, is a small molecule with a molecular weight of 1101.3 g/mol that belongs to cyclic depsipeptide antibiotics that have two quinoxaline moieties. It has a chemical name N, N'-(2,4,12,15,17,25-hexamethyl-11,24-bis(1-methylethyl)-27-(methylthio)-3,6,10,13, 16, 19, 23,26-octaoxo-9,22-dioxa-28-thia-2,5,12,15,18,25-hexaazabicyclo (12.12.3) nonacosane-7,20-diyl)bis (2-quinoxaline carboxamide) (13).

The precursors of the two quinoxaline rings are quinoxaline-2-carboxylic acid and 3-hydroxyquinaldic acid, as shown in Figure 1 (25). Additionally, the octapeptide backbone is a depsipeptide that is divided into two cycles via a thioacetal group (16). The thioacetal is a unique chemical group resulting from the disulfide bridge of triostin A, the precursor of echinomycin, through a methyltransferase and S-adenosyl-L-methionine-dependent pathway (26, 27). A depsipeptide is a peptide that contains one or more ester groups instead of amide groups, giving it both peptide and ester linkages. Echinomycin is a depsipeptide that contains two ester bonds connecting the two amino acids, valine and serine (28). The depsipeptide portion of echinomycin

(octapeptide dilactone) consists of two sets of four amino acids: alanine (L- methyl-Ala), cysteine (methyl -L- Cys), valine (L-Val), and serine (D-Ser), as illustrated in Figure 1 (17, 19). Echinomycin's structural features make it an extremely potent bifunctional DNA intercalator.

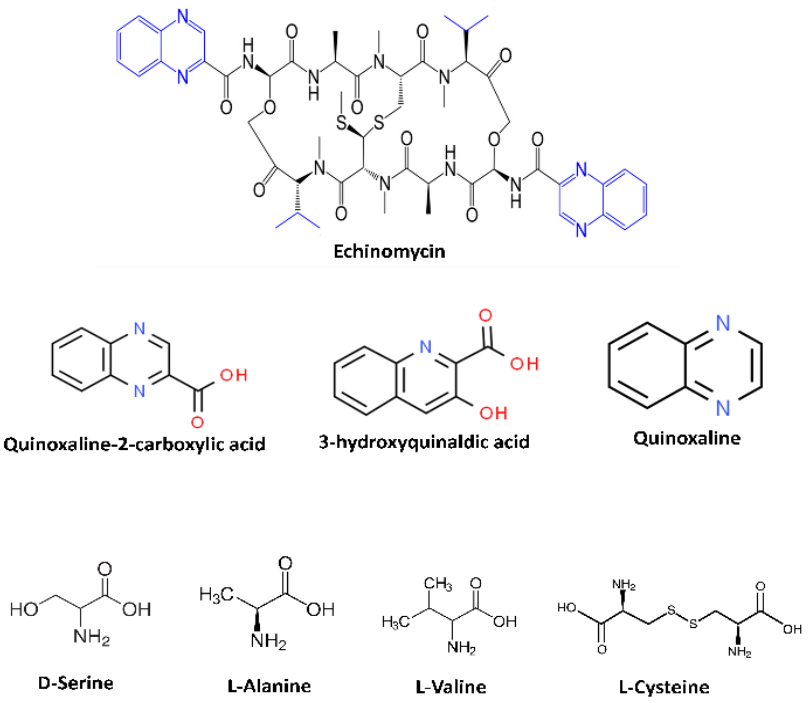

Figure 1: Echinomycin structure and components

Echinomycin is a secondary metabolite originally extracted and purified from Streptomyces echinatus bacteria (29, 30). The core structure of echinomycin is biosynthesized by the non-ribosomal peptide synthetase (NRPS) of this bacterium as part of its defense mechanism against other pathogens (27). The dimerized cyclic peptide core structure is attached to a bicyclic aromatic chromophore quinoxaline. Echinomycin has also been isolated from other bacteria, such as Streptomyces lasaliensis. Mass production of this valuable secondary metabolite for clinical use requires flexible and easily cultivated microorganisms for engineered biosynthesis. Therefore, biosynthesis of echinomycin in Escherichia coli was performed. Firstly, the gene cluster responsible for its biosynthesis from Streptomyces lasaliensis was identified. Then, Escherichia coli was engineered and cultivated under suitable conditions for the large-scale biosynthesis of echinomycin (31, 32).

Sato et al. (2013) successfully reconstituted the biosynthesis pathway using Escherichia coli non-ribosomal peptide synthetase. They declared that echinomycin-engineered biosynthesis by E. coli simplified the confirmation and usage of biosynthetic genes and enzymes, which were identified in other microorganisms that make up the biosynthetic pathways (33). Recently, Kojima et al. performed a retrosynthetic analysis of echinomycin. The study used Pummerer rearrangement of the sulfide moiety to the thioacetal group and rapid

cyclization of the C2-symmetrical depsipeptide ring with a sulfide linkage. They reported the first total synthesis of echinomycin (34).

3.3. Echinomycin mechanism of action

Echinomycin is a DNA bis-intercalator peptide with potent anticancer and antibacterial activity (17, 35). Initially, it was discovered as an antibacterial agent, and ten years later, its antitumor properties were described. In the 1970s, echinomycin's activity as a DNA bis-intercalator was first described, and the DNA binding sequence was identified as CpG. This bifunctional DNA intercalation is due to the presence of two quinoxaline chromophores. In 1974, echinomycin was introduced as the first bis-intercalator (14). Quinoxaline-2-carboxylic acid and 3-hydroxyquinaldic acid moieties in the quinomycin family gave it anticancer activity (16).

Echinomycin can enter the DNA through its major groove and bind in the minor groove (Figure 2) [1]. Echinomycin interacts and forms a stable complex with DNA through three different interactions: Van der Waals forces, hydrogen bonding, and intercalation. The peptide part of echinomycin is essential for strong and specific DNA binding; L-alanine of echinomycin forms a hydrogen bond with the guanine base pair of the 5'-CGTACG-3' sequence in the minor groove (31). Echinomycin was reported to cause a rearrangement of flanking A-T base pairs from Watson-Crick to Hoogsteen pairing when the sequence is 5'-ACGT-3'. NMR studies showed that not all the adjacent AT base pairs are exchanged for Hoogsteen pairing. Binding of echinomycin to [d(ACGTACGT)]2 causes both the internal and terminal AT pairs to be Hoogsteen pairing, while in [d(ACGTATACGT)]2, only the terminal AT pairs is Hoogsteen, and there is no Hoogsteen pairing in [d(TCGAACGT)]2 binding (11, 21).

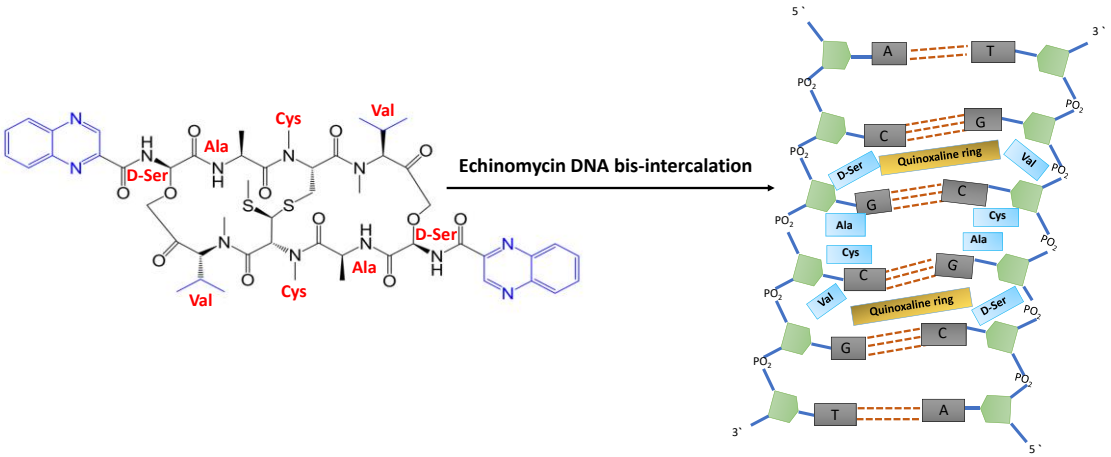

Figure 2: Echinomycin bis-intercalation into DNA

Footprinting method as well as NMR studies for quantitative analysis of echinomycin and DNA interaction revealed that their binding is a cooperative molecular recognition process (19). Cooperative bindging of echinomycin is induced by the DNA disruption cuased by the first echinomycin-DNA complex formed (figure 3). Cooperative binding depends on binding site and its adjacent sequence as in $[d(ACGTACGT)]_2$, $[d(TCGAACGT)]_2$ and $[d(ACGTATACGT)]_2$ parts (17, 21). Dissociation rates of echinomycin from DNA was determined by different kinetic studies. Echinomycin shuffles between different DNA sequences until best binding site is reached. Dissociation of echinomycin is the slowest from its optimal binding site (5'-ACGT-3') (17).

Biologically, DNA intercalating drugs, such as echinomycin, inhibit DNA-dependent RNA synthesis (transcription) and DNA replication. This is due to the inhibition of the separation of the DNA double helix and the prevention of RNA polymerase from binding to the DNA template (5, 7, 36).

White and Phillips (1989) studied the in vitro activity of echinomycin against a variety of RNA polymerases and found that transcription is terminated at the drug binding site. Moreover, a bidirectional transcription footprinting method was developed and found to be more sensitive and specific in determining drug-DNA binding sites than other footprinting methods (37).

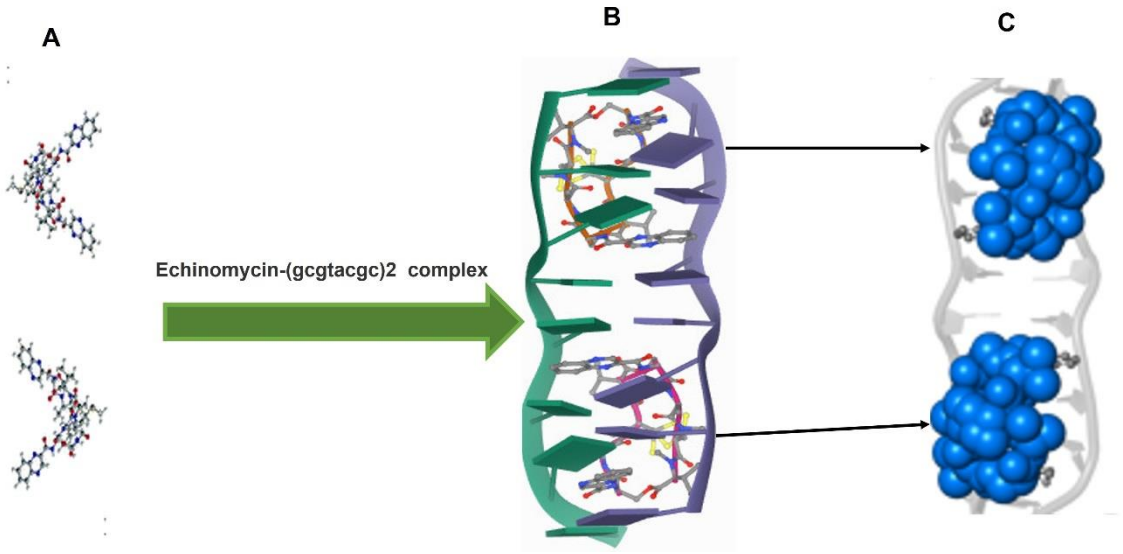

Figure 3: Crystallography mechanism of echinomycin DNA bis-intercalation A) Two molecules of Echinomycin B) show the two molecules intercalate and bind to (gcgtacgc)2 DNA sequence C) Structures of complexes between echinom viewed from the front (1)

Echinomycin specifically inhibits hypoxia-inducible factor-1 (HIF-1), a crucial factor in leukemia cell growth. Due to this inhibition, vascular endothelial growth factor (VEGF) production and the expression of antiapoptotic proteins Bcl-2 and Bcl-xL are reduced, leading to the inhibition of cell proliferation and apoptosis (Figure 4) (38). It also reduces and down-regulates many signaling pathways, including Notch signaling. Recently, echinomycin inhibited HIF-1-facilitated angiogenesis in a mouse model with choroidal neovascularization, which may offer hope for the treatment of neovascular age-related macular degeneration (39).

Interestingly, Park and his colleagues reviewed the toxicological profiles of echinomycin. They suggested that echinomycin could have great potential against human diseases. They demonstrated that echinomycin and its analogues control cellular proliferation through direct action on DNA, certain signaling pathways in mitochondria, and the inhibition of HIF-1α. The attractive echinomycin CG sequence specificity and irreversible binding increase its potency as anticancer therapy with no chemotherapeutic resistance (40).

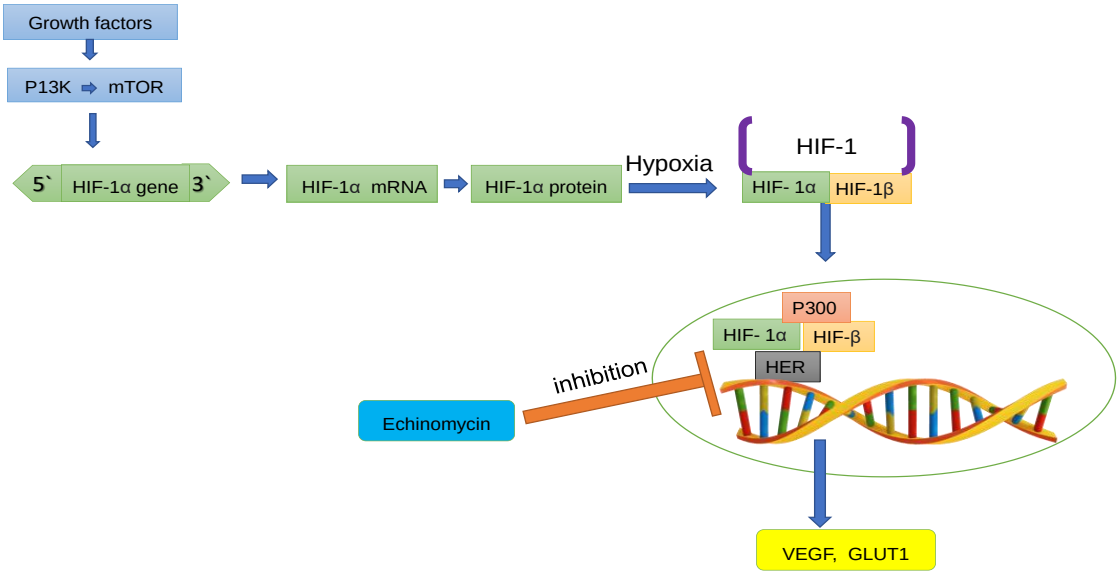

Figure 4: Mechanism of echinomycin inhibition of HIF-1α

Echinomycin has antimicrobial activity: antibacterial, antifungal, and antiviral (14). Echinomycin's inhibition of HIF-1 paved the way for developing treatments against fibrosis, cancer, obesity, infections, and autoimmune diseases (3, 41). Park et al. investigated the antimicrobial activity of echinomycin and compared it with vancomycin. They concluded that echinomycin has the potential to be used against S. aureus, which is resistant to vancomycin (42). The antimicrobial activity of echinomycin was explained by its interaction with bacterial circular DNA. Echinomycin's antibiotic activity was proven to interact selectively with specific DNA sequences in bacterial DNA (43).

4. THERAPEUTIC ACTIVITY

4.1. Echinomycin antibacterial activity

Many in vitro and in vivo assays have demonstrated that echinomycin has excellent activity against S. aureus, including methicillin-resistant Staphylococcus aureus (MRSA), which is equivalent to that of vancomycin, making it a choice for vancomycin-resistant S. aureus species (42). In vivo, echinomycin was more effective than vancomycin in a mouse model against Staphylococcus aureus (44). The in vitro antibacterial assay of echinomycin showed potent activity against several vancomycin-resistant Enterococci (VRE) clinical isolates (45). The good antibacterial properties of echinomycin against both Gram-positive and Gram-negative bacteria have encouraged efforts to synthesize and discover new quinoxaline derivatives. Echinomycin's activity against biofilm-forming MRSA and vancomycin-resistant Enterococcus faecalis was tested, and its minimum inhibitory concentrations (MIC) were found to be 0.03 µM against Staphylococcus aureus and 0.01 µM against Enterococcus faecalis (46).

4.2. Echinomycin antitumor activity

Recently, there has been a growing necessity and effort to develop new anticancer drugs to combat cancer resistance. In vitro, echinomycin has exhibited cytotoxic activity across diverse cell lines (21). Echinomycin advanced to phase I and II clinical trials for various cancer diseases, including endometrial carcinoma, ovarian cancer, soft tissue sarcoma, and others (Table 1) (6, 9, 47). However, the results demonstrated low or no efficacy, accompanied by serious side effects such as nausea, vomiting, reversible liver enzyme abnormalities, and allergic reactions (48).

Table 1: Timeline and evolution of echinomycin screening and investigation of therapeutic activity

Disease	Investigation	Formulation	Comments	Reference
Different tissue types/ Normal and cancer	*In vitro/* tumour colony-forming units	Echinomycin in ethanol	50% survival colony	(49)
B16 melanoma, and the P388 leukemia	Preclinical Murine/ dogs	Conventional formulation/ CrEL-based	Toxicity study /LD50	(50)
Advanced carcinoma	Phase I	Conventional formulation	Toxicity study/dose escalation	(51)
Advanced cancer	Phase I	Conventional formulation	Toxicity study/dose escalation	(52)
Squamous-cell carcinoma of the cervix.	Phase II	Conventional formulation	7% response	(47)
Metastatic cervix carcinoma	Phase II	Conventional formulation	No response	(53)
Advanced Ovarian cancer	Phase II	Conventional formulation	9% response	(47)
Advanced colorectal cancer	Phase II	Conventional formulation	No response	(54)
Stage IV recurrent or inoperable breast cancer	Phase II	Conventional formulation	4.6% response	(55)
Recurrent and metastatic nonsquamous cell carcinoma of the cervix:	Phase II	Conventional formulation	5.6% response	(48)
Recurrent and metastatic endometrial carcinoma	Phase II clinical trial	Conventional formulation	5% response	(9)
Renal cell carcinoma	Phase II clinical trial	Conventional formulation	5.6% response	(56)
Recurrent colorectal cancer	Phase II clinical trial	Conventional formulation	10% response	(57)
Soft tissue carcinoma	Phase II	Conventional formulation	No response	(6)
Metastatic Non-small Cell Lung Carcinoma	Phase II	Conventional formulation	5% response	(58)
leukaemia P388, melanoma B 16 and gastric SNU-16	*In vitro/ in vivo*	modified-echinomycin/ S-methylated sulfonium perchlorate of echinomycin	IC50 8-9 µg/ml For leukaemia P388, melanoma B 16 while gastric SNU-16 quite different need more studies	(59)
Xenopus sperm chromatin and cervical HeLa- S3cell nuclei *in vitro*	*In vitro: Xenopus sperm chromatin and HeLa cell nuclei / in vivo: embryos from Xenopus laevis*	In methanol and stored at –20°C	Anti- proliferative effects by inhibition of chromosomal DNA replication and embryonic development	(60)
HT-29 cells colorectal cancer cell line	*In vitro*	Organic solution	Apoptotic MAP kinases signalling pathways	(40)
U251 human glioma cells and MCF-7 cells	*In vitro*	Organic solution	Inhibited hypoxic induction of luciferase in cells and VEGF mRNA expression	(7)
Vancomycin-resistant enterococci	*In vitro*	Organic solution	MIC 0.125 µg/ml	(61)
Restenosis and thrombosis of echinomycin-eluting stents	*In vivo/pigs*	echinomycin-eluting stents topcoated with a hydrophobic heparin-polymer	effectively reduced both restenosis and thrombosis	(62)

Disease	Investigation	Formulation	Comments	Reference
Liver cancer HepG2 and cervical Hella cells	*In vitro*	Organic solution	dual effect on HIF-1 activity under normoxic and hypoxic conditions,	(10)
Clinical isolates of Staphylococcus aureus	*In vitro/ in vivo*	Organic solution	MIC 0.125 µg/ml	(42)
Biofilm-forming strains of Staphylococcus aureus Enterococcus faecalis.	*In vitro*	Organic solution	MIC 0.01- 0.03µM	(46)
Glioma stem cells lymphoma myeloid leukaemias (AML)	*In vitro*	Organic solution	regulate the tumorigenic capacity	(3)
Ovarian ovulation in mammalians	*In vivo*	Conventional formulation	Regulating gonadotropin-induced mammalian ovulatory process in vivo.	(63)
Leukaemia Cells lymphoma myeloid leukemia (AML)	*In vitro*	Organic solution	suppresses NOTCH1 signalling and suppress growth	(38)
Heterotopic ossification	*In vivo*	Echinomycin was diluted in dimethyl sulfoxide (DMSO) and administered subcutaneously	highly significant reduction in the bone volume	(64)
FKBP12 protein.	*In silico Docking*	Computer aid	echinomycin may have a double impact on HIF direct inhibition and through mTOR	(65)
Relapsed acute myeloid leukaemia without	Preclinical	Low dose echinomycin Organic solution	40% to 60%	(66)
Three pancreatic cancer cell lines, MiaPaCa-2, BxPC-3 and PanC-1/ tumour xenograft	*In vitro/ in vivo*	Quinomycin in 10% FBS	Significant inhibition of proliferation and colony formation in pancreatic cancer cell lines and tumour xenograft growth	(67)
Follicular Development in the Ovary of Postnatal Rats/ Granulosa cell culture	*In vitro/ in vivo*	Organic solution	Inhibition of follicular development	(68)
Endometriosis	Ectopic endometriotic tissues	Organic solution	100 nM decrease VEGF production	(8)
Adipogenesis in 3T3-L1 cells/white adipose tissue	*In vitro/ in vivo*	Organic solution	inhibited adipogenesis and body weight gain in high fat diet mice	(69)
Breast cancer	*In vitro/ in vivo*	Liposomal formulation	1200µg/m^2	(70)
H6OHDA induced Parkinson's disease model using SH-SY5Y human neuroblastoma	*In vitro/ In vivo*	Organic solution	Notch signalling pathway was decelerated and b-catenin stabilization was increased.	(71)

Disease	Investigation	Formulation	Comments	Reference
Glioblastoma	*In vitro*	Thermosensitive Liposomal- γ cyclodextrin formulation	IC50/1nM	(24)
Solid tumors/ metastatic breast cancer	*in vitro/ In vivo*	Liposomal formulation	Increase therapeutic index	(72)
Chromosome-negative myeloproliferative neoplasms	*Ex vivo* patient samples and *in vitro 32D cells*	Organic solution	Selectively decreased growth of JAK2V617F cells at 1 nM	(73)
Metastases of triple-negative breast cancer	*In vitro/ in vivo*	Liposomal formulation	Effective and less toxic than conventional formulations	(74)
Chemo-resistant Pancreatic Cancer	*in vivo*	syndecan-1 actively targeted nanoparticle	Autophagy-Mediated Death	(75)
Regresses tumour growth of lung cancer and lymphoma	*In vitro*	Organic solution	Cells were degraded through proteasome dependent pathways	(76)
Age-related Macular degeneration	*In vitro/ in vivo*	Organic solution	Significantly decreased vascular lesion	(39)
Breast cancer and Lung cancer	*In vitro*	Antinucleon aptamer targeted pH-sensitive- γ cyclodextrin- liposomes	IC50 MCF7, 0.46 nM MDA-MB-231, 0.18 Nm A549 0.92nM	(77)

However, Huang et al. investigated the antitumor activity of echinomycin against lung cancer and lymphoma in vitro and in vivo. They proposed that echinomycin instantaneously inhibited MYC and HIF1α, leading to a reversal in tumor cell growth (76). In May 2015 and 2017, the OncoImmune company manufactured echinomycin and received orphan drug designations for treating myeloid leukemia (AML) and graft-versus-host disease (GVHD) in the U.S.A., respectively.

5. Echinomycin drug delivery and dosage forms

The peptide nature and extreme lipophilicity constitute the main obstacles to properly formulate echinomycin into a pharmaceutical dosage form (15). Consequently, echinomycin was formulated as a conjugate with Cremophor EL, a non-ionic emulsifier produced by the reaction of ethylene oxide and castor oil to solubilize hydrophobic drugs (78). In many drug formulations, such as echinomycin, Cremophor EL has been known to cause allergic and hypersensitivity reactions (78). It is believed that the use of Cremophor EL was one of the factors that led to the discontinuation of echinomycin clinical trials (72). Recently, nanoparticle drug delivery systems (79) have become suitable for all compounds with low water solubility and high toxicity (80).

Wang et al. developed a liposomal formulation of echinomycin. The hydrophobic echinomycin was encapsulated into the liposome bilayer, and they proposed that the new formulation enhanced the drug's physicochemical properties and decreased its toxicity (72). In another study, echinomycin was complexed with γ-Cyclodextrin, and the inclusion complex was encapsulated inside PEGylated thermosensitive liposomes and tested for their cytotoxicity using a Glioblastoma cell line (81). Meanwhile, Bailey et al. studied the activity of the liposomal echinomycin formulation on triple-negative breast cancer in vitro and in vivo. They reported that liposomal echinomycin is a more potent inhibitor of HIF-1α transcriptional activity in primary and metastasized cells in vivo (74).

In a further study, liposomes encapsulating echinomycin were fabricated using a PEGylated phospholipid, a neutral phosphoglyceride, and a sterol for treating patients who show

overexpression of HIF-1α and/or HIF-2α. Additionally, echinomycin PEGylated liposomal formulation has promising potential for the treatment of many diseases, including proliferative diseases, autoimmune diseases, and graft-versus-host disease (7).

Another pH-sensitive liposomal formulation functionalized with an antinucleon aptamer was tested in vitro using various cancer cell lines. Aptamer-targeted pH-sensitive PEGylated liposomes were designed, formulated, and fully characterized. These liposomes remained stable at physiological pH and released their payload at low pH. These innovative liposomes exhibited excellent selectivity and cytotoxicity against three cancer cell lines: MCF7, MDA-MB-231 breast cancer, and A549 lung cancer cell lines (77).

6. Conclusion and future insights

Echinomycin possesses a unique structure, an intriguing mechanism of action, and promising potential as both an antimicrobial and anticancer therapy. Nanoliposome formulations have demonstrated enhanced potency and selectivity while mitigating side effects. Researchers will continue their exploration of echinomycin, aiming to address the main challenges associated with this promising drug. These challenges encompass various aspects, including the development of cost-effective production methods and the improvement of its bioavailability.

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Jordan Journal of Pharmaceutical Sciences, Volume 16, No. 3, 2023

عقار الإكينوماسين: رحلة التحديات

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ملخص

الإكينومايسن هو مركب طبيعي تم اكتشافه وعزله من البكتيريا وتم تقديمه كمضاد حيوي وعلاج مضاد للسرطان. ومع ذلك، فقد فشل سريريًا بسبب التركيبات غير الصحيحة وسرعة تحطيمه في الجسم. بعد التجارب السريرية غير الناجحة، تم وقف التجارب السريرية عليه في الآونة الأخيرة، أعطت آلية جديدة بعض الأمل في إحياء مادة الاكينوميسين كمثبط للعامل ((HIF-1 المحرض لنقص الأكسجين، و في عام 2015 إكينوميسين وصف لعلاج ابيضاض الدم النخاعي الحاد في الولايات المتحدة الأمريكية. علاوة على ذلك، أتاحت التطورات في أنظمة توصيل الأدوية آفاقًا جديدة للتغلب على مشكلات تركيبة الإكينومايسين واستكشاف المزيد من الفوائد العلاجية. توضح هذه المراجعة تفاصيل رحلة إيكينومايسين إلى جانب التحديات الرئيسية لهذا الدواء الفعال وتقدم رؤى حول التطبيقات السريرية المحتملة في المستقبل.

الكلمات الدالة: إكينومايسين، روابط مستهدفة، ببتيدات دورية، مضاد حيوي كينوكسالين، مقسم ثنائي الحمض النووي.

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Exploration of Anthelmintic, Blood Coagulant, Diuretic and Laxative Activities of Different Solvent Fractions of Flagellaria Indica Leaves

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ABSTRACT

Flagellaria indica (Family: Flagellariaceae) is a common climbing plant found primarily in tropical regions of many countries. The plant has various traditional uses, although most of them lack scientific published reports. The crude ethanolic extract of F. indica leaves was fractionated based on polarity using water, ethyl acetate, and n-hexane. Biological screening was conducted on the anthelmintic, blood coagulation, diuretic, and laxative activities of the water, ethyl acetate, and n-hexane fractions of F. indica leaves. In the anthelmintic test, the n-hexane fraction showed a moderate effect with paralysis times of 16.79 and 13.62 minutes and death times of 27.34 and 21.81 minutes, respectively, at doses of 25 and 50 mg/mL.In the blood coagulant test, only the water fraction showed a notable effect. The clotting times were 4.33, 6.02, 7.68, and 8.32 minutes, respectively, at doses of 200, 100, 50, and 25 mg/mL. Diuretic activity was performed to determine the increase in the volume of excreted urine, and electrolyte analysis of urine was performed to determine pH, density, conductance, and Na+, Cl-, K+ levels, as well as natriuretic, kaliuretic, saluretic, and CAI indexes. The ethyl acetate fraction showed better diuretic activity than the n-hexane fraction, while the water fraction did not reveal a notable diuretic effect. The Na+, Cl-, K+, natriuretic, and saluretic indexes were found satisfactory in the ethyl acetate fraction, and the CAI index was better in the n-hexane fraction. In the laxative test, the n-hexane fraction showed the best laxative properties, with an increase in stool weight of 38% and 54% at doses of 250 and 500 mg/kg, respectively. These results suggest that different fractions of F. indica leaves contain distinct phytochemicals that may be responsible for these biological effects. The isolation of bioactive compounds could help justify its traditional uses in modern medicine.

Keywords: *Flagellaria indica*, Anthelmintic, Blood Coagulant, Diuretic and Laxative activities.

Abbreviations: gm, gram; mg, milligram; kg, kilogram; mg/kg, milligram/kilogram; bw, bodyweight; min, minutes; mm, millimeter; cm, centimeter, L, liter, mL, milliliter; µL, microliter; ICDDRB, International Center for Diarrhoeal Disease Research, Bangladesh; SD, Standard Deviation; ANOVA, Analysis of Variance, WHO, World Health Organization, AEC, Animal Ethics Committee; CAI, Carbonic Anhydrase Inhibitory.

INTRODUCTION

Our universe is the ultimate reservoir of all living creatures. Medicinal plants are the best gift of nature for human survival against different types of physical disorders. Human beings have been solely dependent on medicinal plants since ancient times. Flagellaria indica is a common plant from the Flagellariaceae family (Figure 1). This climbing plant is widely located in many subtropical and tropical areas of Polynesia, South-East Asia, and Australia. The plant is also well-known by many local names, such as rotan tikas, Bon chanda, whip vine, hell tail, supplejack, etc. In Bangladesh, this plant is found in different parts of the Sundarbans. It can often grow up

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to 15 meters tall, with stems that are 15 mm in diameter. Its leaves are 10-40 cm long and 5-20 mm wide, forming a coiled apex that helps the plant climb upward. The white flowers have a pleasant fragrance and are 10-25 cm long. The green fruits are 5 mm in diameter and are inedible. This plant has various local uses by people from different areas of the world. The leaves are used to treat bacterial infectious diseases, coughs, helminthiasis, vomiting, and they also have diuretic effects. The Murut tribe people use its boiled leaves for bathing to treat semi-paralytic disorders. Chopped small stems are used as traditional medicine to treat diarrhea, stomach pain, and cholera. The stem of this plant is also reported to be used as a contraceptive in limited cases. A decoction of the leaves is beneficial for treating asthma, wheezing, and fevers.[1, 2]

Karmakar et al. (2021) reported the presence of various phytochemical groups such as reducing sugars, terpenoids, tannins, saponins, steroids, flavonoids, gums, alkaloids, etc. They also conducted tests for analgesic, antidiarrheal, antihyperglycemic, and cytotoxic effects using its leaves.[2] Gnanaraj et al. (2015) reported on the antioxidant properties of various plant parts [1] Gnanaraj et al. (2016) reported on the hepatoprotective effects of the plant's leaves[3]. After reviewing the traditional uses and reported activities of this plant, our objective was to conduct additional biological tests that had not been previously investigated. To achieve this, we conducted experiments to assess the anthelmintic, diuretic, blood coagulation, and laxative effects of various fractions of F. indica leaves.

Figure 1: *Flagellaria indica* leaves and fruits

MATERIALS AND METHODS

Plant collection and identification

In July 2017, fresh leaves of F. indica were collected from the riverside area of the Mongla range in the Sundarbans, the world's largest mangrove forest. Great care was taken to ensure there were no contaminants. The collected plant material was then identified by experts from Khulna University's Department of Forestry and Wood Technology, and a voucher number (KUPL-302) was assigned for future reference. The harvested leaves were dried in the shade for 45 days, with strict avoidance of direct sunlight exposure. After the drying period, the leaves were ground into a fine powder. A total of 250 grams of powdered material was macerated with 1 liter of 96% ethanol, and this mixture was sealed in a glass bottle for three weeks. During this period, the mixture was periodically agitated using a glass rod to ensure proper mixing. Subsequently, the mixture was filtered to obtain 7.6 grams of crude gum extract. The crude extracts were then fractionated using water, ethyl acetate, and n-hexane, resulting in the isolation of 3.1 grams, 1.9 grams, and 2.3 grams of extract for the water, ethyl acetate, and n-hexane fractions, respectively.

Animals

For pharmacological tests, fresh male Swiss albino mice, aged 3-5 weeks and with an average body weight of 25-35 grams, were procured from the Animal Resources Division of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR, B). These mice were accommodated in the animal facility at the Laboratory of Pharmacy, Khulna University, where optimal laboratory conditions were maintained (50-60% relative humidity, 25-30°C, and a 12-hour light/dark cycle). They were provided with rodent food. To ensure their acclimatization to the laboratory environment, the mice were given a period of 14 days before commencing any experiments. All bioassays performed on the mice adhered to the ethical standards outlined by the Khulna University Animal Ethics Committee (AEC), Bangladesh [Reference: KUAEC-2020/12/25].

Chemicals

All laboratory-grade reagents required for conducting the biological experiments, such as ethanol (Merck, India), n-Hexane (Merck, India), Ethyl acetate (Loba, India), and Tween 80 (Merck, India), were used. Albendazole, phytomenadione, and frusemide were procured from Square Pharmaceuticals Limited, Bangladesh, while bisacodyl was obtained from Opsonin Pharmaceuticals Limited, Bangladesh.

Anthelmintic activity Assay

The anthelmintic impact of F. indica was assessed using the method described by Saha et al. (2021) and Akter et al. (2020) to measure its lethal effect on Paramphistomum cervi. Live P. cervi specimens were collected from freshly slaughtered cattle at a local abattoir in Gollamari, Khulna. Ten milliliters of 25 mg/mL and 50 mg/mL concentrations of n-Hexane, ethyl acetate, and water fractions of F. indica were prepared and placed in separate petri dishes. Albendazole was used as the standard drug at a concentration of 15 mg/mL, and 0.1% Tween 80 solutions were also prepared, with 10 mL of each placed in separate petri dishes. In each petri dish, six P. cervi specimens were introduced. Subsequently, the paralysis time (defined as no movement except for slight twitching after vigorous shaking) was recorded using a stopwatch. Additionally, the time of death (defined as no movement after vigorous shaking and immersion in 50º C water) was also recorded.

Blood coagulant Activity Assay

The blood coagulation activity of various fractions of F. indica extract was assessed using the method described by Ikese et al. (2015).[6] Five healthy human volunteers agreed to provide blood for this experiment. Fresh human blood was collected using sterile syringes.

n-Hexane, ethyl acetate, and water fractions of F. indica were prepared at concentrations of 25, 50, 100, and 200 mg/mL. Phytomenadione was used as the standard drug and was prepared at concentrations of 1.25, 2.5, 5, and 10 mg/mL. One milliliter of freshly collected blood was mixed with 100 µL of the above-prepared concentrations of Phytomenadione and different fractions of F. indica in separate labeled test tubes. The test tubes were immediately placed in a water bath containing water at 37º C (normal human body temperature), and the time for clot formation or gel-like substance formation was recorded using a stopwatch. Every 15 seconds, each test tube was tilted to check for the formation of clotting. Finally, the coagulation time was recorded.

Diuretic activity Assay

Diuretic assay was conducted following an established protocol adopted by Mamun *et al.* (2003) and Mekonnen *et al.* (2010).[7, 8] Mice for the study were divided into several groups, each containing 5 mice. The mice were fasted for 18 hours prior to the experiment and were pre-treated with 0.9% NaCl saline solution. In the experiment, the standard group was orally administered furosemide at a dose of 10 mg/kg body weight, while the control group received pre-treated saline solution. The test groups were given oral doses of 250 and 500 mg/kg of the water, ethyl acetate, and n-Hexane fractions of F. indica extract. The solutions were prepared so that each mouse received a 2 mL solution of the respective doses. Subsequently, these mice were placed in metabolic cages according to their respective groups. Urine excreted by the mice was collected and quantified hourly for 6 hours after the experiment was conducted. The collected urine was stored under refrigeration at -20º C for future analysis.

The diuretic effect of F. indica's different fractions was measured using the following equations:

Urinary discharge = (Total urine amount (Vo) / Total fluid administered (Vi)) x 100

The diuretic activity was calculated by dividing the urinary excretion of the test group by the urinary excretion of the control group. The diuretic activity was then determined by comparing the diuretic activity of the test group to that of the control group.

As soon as we collected the urine, it was measured for pH using the D-50 Series Handheld Water Quality Meters by Horiba Scientific. The density of urine was determined by measuring the volume and weight of the urine. Sodium (Na+) and potassium (K+) ion concentrations were measured in mEq/L using a Jenway PFP7 Flame Photometer with a 100 times diluted solution. Chloride (Cl-) ion concentration was assessed through direct titration with a 1% $AgNO_3$ solution using potassium chromate (5%) as an indicator. After measuring the ion concentrations, the natriuretic (Na+/K+), saluretic (Na+/Cl-), carbonic anhydrase inhibitory (CAI) activity [(Cl-/(Na++K+)], and kaliuretic (K+/Na+) values were calculated. Finally, the Na+, K+, Cl-, saluretic, natriuretic, kaliuretic, and CAI indices were determined by comparing the values in the test group with those in the control group[9]

Laxative activity Assay

The laxative effect of the fractions of F. indica extract was evaluated using the method described by Capasso (1986) and Akter et al. (2022).[10,11] The test mice (25-30 gm body weight) were divided into several groups, each consisting of five mice. Prior to the test, the mice were fasted for 12 hours and only water was provided during this period. In the control group, mice were treated with normal saline (0.9% NaCl), and the positive control group received bisacodyl at a dose of 10 mg/kg. In the test groups, mice were administered doses of 250 and 500 mg/kg body weight of n-Hexane, ethyl acetate, and water fractions of F. indica extract. The mice were then placed in metabolic cages according to their respective groups for the next 16 hours, during which no food or water was

provided. After this time, the excreted feces from the mice were collected and weighed.

Statistical data analysis

One-way ANOVA analysis was conducted using Dunnett's t-test ($p < 0.05$, versus control). Pairwise comparisons were carried out using the Post-hoc Tukey test ($p < 0.05$, versus standard/extract). To analyze the data, the IBM SPSS program (version 25.0) from IBM Corporation, New York, USA, was utilized.[12]

RESULTS

Anthelmintic activity Assay

In evaluating the anthelmintic assay of different fractions of F. indica, significant paralysis and death times of P. cervi were observed (Table 1). Among the three fractions of F. indica, the n-Hexane fraction showed the best anthelmintic activity, causing the least time for paralysis and death of the nematodes. Conversely, the water fraction showed comparatively poor anthelmintic activity across the tested doses. The results obtained demonstrated a dose-dependent effect.

Table 1: Representation of anthelmintic effects of different fractions of *F. indica* extract

Tested group	Dose (mg/mL)	Mean paralysis time (min)	Mean death time (min)
Negative control (0.9% NaCl)	------	------	------
Standard (Albendazole)	15	6.95 ± 0.44^{abcdef}	14.71 ± 0.75^{abcdef}
n-Hexane fraction	25	$16.79 \pm 0.99^{\theta bcdef}$	$27.34 \pm 0.76^{\theta bcef}$
n-Hexane fraction	50	$13.62 \pm 0.76^{\theta acdef}$	$21.81 \pm 1.13^{\theta acdef}$
Ethyl Acetate fraction	25	$20.99 \pm 1.13^{\theta abde}$	$30.48 \pm 0.7^{\theta abd}$
Ethyl Acetate fraction	50	$18.70 \pm 0.79^{\theta abcef}$	$28.17 \pm 0.78^{\theta bcef}$
Water fraction	25	$22.17 \pm 1.18^{\theta abcdf}$	$31.43 \pm 0.87^{\theta abdf}$
Water fraction	50	$20.26 \pm 1.18^{\theta abde}$	$30.33 \pm 1.21^{\theta abde}$

Data are plotted as average of six (06) replicates ± SD (standard deviation);
$^{\theta}$ $p < 0.05$ vs. Albendazole 15 mg/mL; ^{a} $p < 0.05$ vs. *n*-Hexane fraction 25 mg/mL; ^{b} $p < 0.05$ vs. *n*-Hexane fraction 50 mg/mL; ^{c} $p < 0.05$ vs. Ethyl Acetate fraction 25 mg/mL; ^{d} $p < 0.05$ vs. Ethyl Acetate fraction 50 mg/mL; ^{e} $p < 0.05$ vs. Water fraction 25 mg/mL; ^{f} $p < 0.05$ vs. Water fraction 50 mg/mL; (pair-wise comparison by Post Hoc Tukey test)

Blood coagulation activity Assay

In the blood coagulation test, various concentrations of the standard (Phytomenadione) and test groups (n-Hexane, ethyl acetate, and water fractions of F. indica extract) were utilized. The average blood coagulation times for the different tested samples are listed in Table 2. Among the tested groups, only the water fraction showed some blood coagulation effect, while the ethyl acetate and n-hexane fractions did not exhibit any notable blood coagulant effect.

Table 2: Representation of Blood Coagulation Effect of different fractions of *F. indica* extract

Tested group	Dose (mg/mL)	Average coagulation time
Negative control	------	8.87 ± 0.00 [1 2 3 4 a e f g i j k l]
Standard (Phytomenadione)	10	2.52 ± 0.09* [2 3 4 a b c d e f g h i j k l]
Standard (Phytomenadione)	5	4.8 ± 0.23 [*1 3 4 a b c d e f g h I j k l]
Standard (Phytomenadione)	2.5	5.73 ± 0.14* [1 2 4 a b c d e f g h I k l]
Standard (Phytomenadione)	1.25	7.77 ± 0.15 [*1 2 3 a b c d e f h i j l]
n-Hexane fraction	200	8.39 ± 0.2* [1 2 3 4 b e f i j k]
n-Hexane fraction	100	8.98 ± 0.14 [1 2 3 4 a e f h i j k l]
n-Hexane fraction	50	8.82 ± 0.22 [1 2 3 4 e f g i j k l]
n-Hexane fraction	25	8.59 ± 0.57 [1 2 3 4 e f g i j k]
Ethyl acetate fraction	200	6.78 ± 0.14* [1 2 3 4 a b c d g h i j k l]
Ethyl acetate fraction	100	7.22 ± 0.19* [1 2 3 4 a b c d g h i j k l]
Ethyl acetate fraction	50	8.12 ± 0.09 [*1 2 3 b c d e f h i j]
Ethyl acetate fraction	25	8.74 ± 0.09 [1 2 3 4 e f g i j k]
Water fraction of *F. indica*	200	4.33 ± 0.16 [*1 2 3 4 a b c d e f g h j k l]
Water fraction of *F. indica*	100	6.02 ± 0.13 [*1 2 3 a b c d e f g h i k l]
Water fraction of *F. indica*	50	7.68 ± 0.16 * [1 2 3 4 a b c d e f h i j l]
Water fraction of *F. indica*	25	8.32 ± 0.08 [*1 2 3 4 b c e f i j k]

Data are plotted as average of five replicates ± SD (standard deviation); * $p < 0.05$ vs. Control (Dunnett's t test); [1] $p < 0.05$ vs. Phytomenadione 10 mg/mL; [2] $p < 0.05$ vs. Phytomenadione 5 mg/mL; [3] $p < 0.05$ vs. Phytomenadione 2.5 mg/mL; [4] $p < 0.05$ vs. Phytomenadione 1.25 mg/mL; [a] $p < 0.05$ vs *n*-Hexane fraction 200 mg/mL; [b] $p < 0.05$ vs. *n*-Hexane fraction 100 mg/mL; [c] $p < 0.05$ vs. *n*-Hexane fraction 50 mg/mL; [d] $p < 0.05$ vs. *n*-Hexane fraction 25 mg/mL; [e] $p < 0.05$ vs. Ethyl Acetate fraction 200 mg/mL; [f] $p < 0.05$ vs. Ethyl Acetate fraction 100 mg/mL; [g] $p < 0.05$ vs. Ethyl Acetate fraction 50 mg/mL; [h] $p < 0.05$ vs. Ethyl Acetate fraction 25 mg/mL; [i] $p < 0.05$ vs. Water fraction 200 mg/mL; [j] $p < 0.05$ vs. Water fraction 100 mg/mL; [k] $p < 0.05$ vs. Water fraction 50 mg/mL; [l] $p < 0.05$ vs. Water fraction 25 mg/mL (Pair-wise comparison by Post Hoc Tukey test)

Diuretic activity Assay

In the diuretic test, the urinary volume, urinary excretion, diuretic activity, and diuretic effects are depicted in Figures 2, 3, 4, and 5, respectively. The pH, density, conductivity, urinary volume, and diuretic index of mice in different groups are listed in Table 3. Tables 4 and 5 show the effects of various fractions of F. indica on electrolyte excretion in mouse urine after 6 hours of treatment.

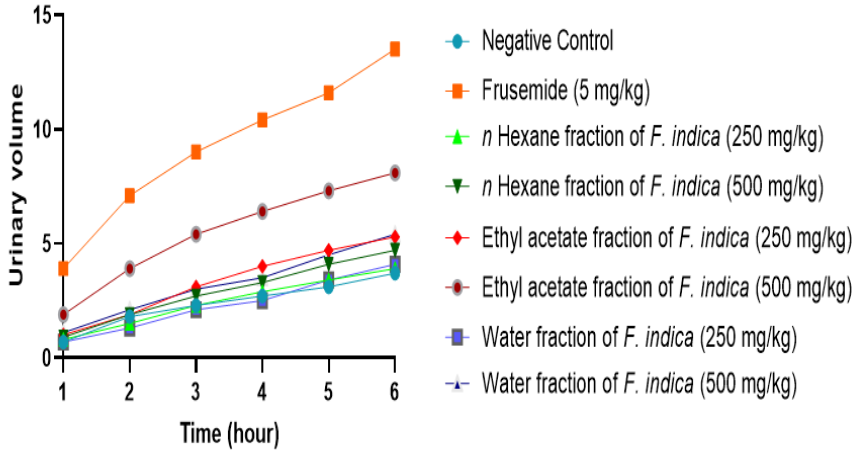

Figure 2: Urinary volume of frusemide and different fractions of *F. indica* leaves

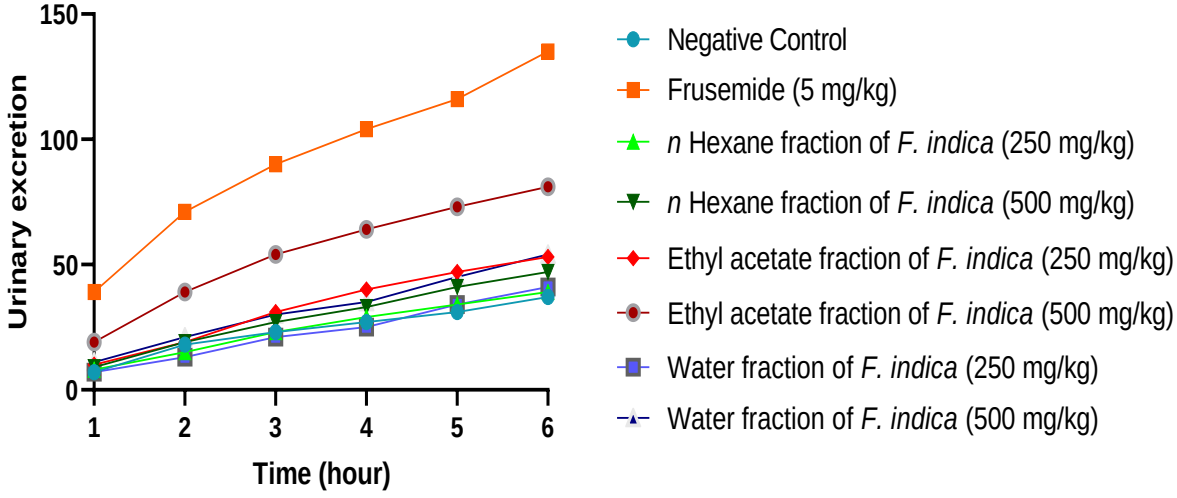

Figure 3: Urinary excretion of frusemide and different fractions of *F. indica* leaves

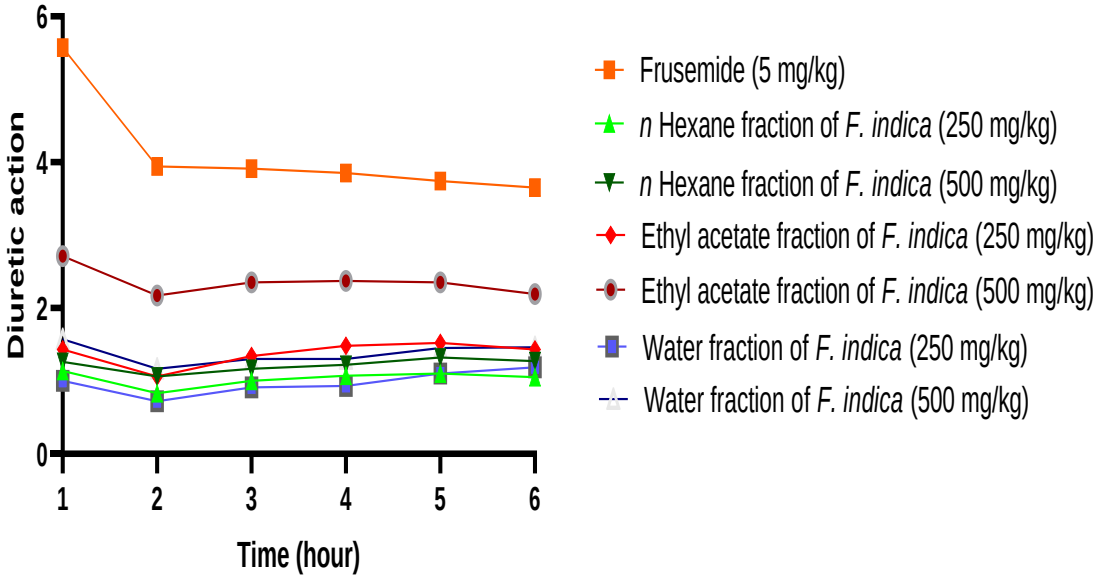

Figure 4: Diuretic action of frusemide and different fractions of *F. indica* leaves

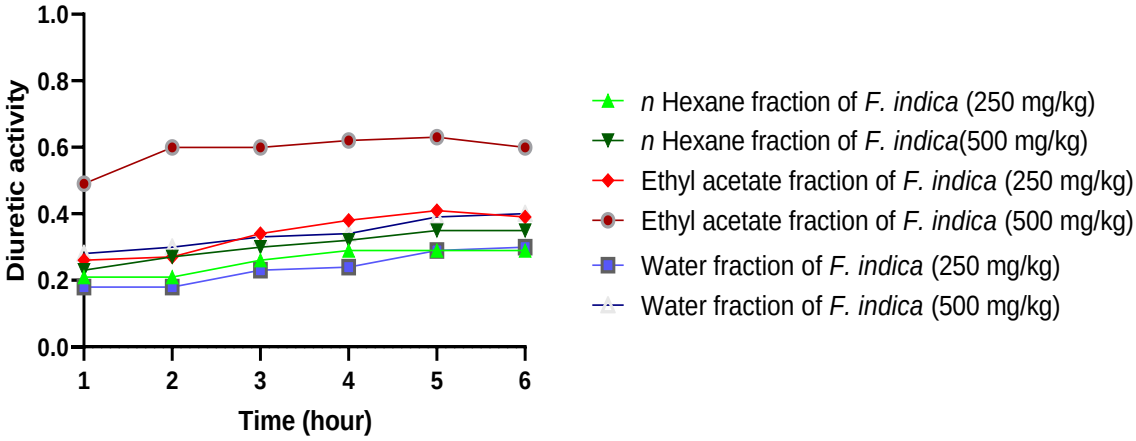

Figure 5: Diuretic activity of different fractions of *F. indica* leaves

Table 3: Impact of various fractions of *F. indica* on pH, density, conductivity, urinary volume and diuretic index of mice urine after 6 hours of medication in the diuretic assay

Tested group	pH	Density (gm/mL)	Conductivity (mS/cm)	Urine Volume (mL/6h)	Diuretic index
Control	7.12 ± 0.02	0.93 ± 0.01	13.33 ± 0.04	3.7	1
Frusemide (5 mg/kg)	7.42 ± 0.03	0.98 ± 0.02	12.32 ± 0.07	13.5	3.65
n-hexane (250 mg/kg)	7.88 ± 0.02	0.89 ± 0.03	14.43 ± 0.08	3.9	1.05
n-hexane (500 mg/kg)	7.78 ± 0.03	0.91 ± 0.02	18.86 ± 0.33	4.7	1.27
Ethyl acetate (250 mg/kg)	7.68 ± 0.02	0.84 ± 0.03	17.54 ± 0.41	5.3	1.43
Ethyl acetate (500 mg/kg)	7.63 ± 0.03	0.89 ± 0.11	23.32 ± 0.22	8.1	2.19
Water (250 mg/kg)	7.56 ± 0.03	0.93 ± 0.05	14.52 ± 0.53	4.1	1.11
Water (500 mg/kg)	7.52 ± 0.02	0.88 ± 0.10	15.11 ± 0.22	5.4	1.46

Table 4: Effect of different fractions of *F. indica* on electrolyte excretion of mice urine after 6 hours of medication in diuretic assay

Tested group	Na^+ (mEq/L)	K^+ (mEq/L)	Cl^- (mEq/L)	Cumulative Saluretic (Na^++Cl^-)	Natriuretic (Na^+/K^+)	Kaliuretic (K^+/Na^+)	CAI [Cl^-/ (Na^+ +K^+)]
Control	83.91 ± 0.00	71.90 ± 0.00	85 ± 2.5	168.91	1.17	0.86	0.55
Frusemide (5 mg/kg)	149.22 ± 0.00	141.49 ± 0.00	120 ± 2.5	269.22	1.05	0.95	0.41
n-hexane (250 mg/kg)	67.57 ± 16.34	89.29 ± 5.8	81.25 ± 1.25	148.82	0.76	1.32	0.52
n-hexane (500 mg/kg)	116.61 ± 0.00	106.72 ± 0.00	91.25 ± 1.25	207.86	1.09	0.92	0.41
Ethyl acetate (250 mg/kg)	100.26 ± 16.35	112.52 ± 5.8	105 ± 2.5	205.26	0.89	1.12	0.49
Ethyl acetate (500 mg/kg)	165.57 ± 16.35	147.31 ± 17.41	118.75 ± 1.25	284.32	1.12	0.89	0.38
Water (250 mg/kg)	83.91 ± 0.00	95.08 ± 0.00	98.75 ± 1.25	182.66	0.88	1.13	0.55
Water (500 mg/kg)	100.26 ± 16.35	141.49 ± 11.59	106.25 ± 1.25	206.51	0.71	1.41	0.44

Table 5: Effect of different fractions of *F. indica* on electrolyte excretion of mice urine after 6 hours of medication in diuretic assay (Cont.)

Treatment group	Na^+ index	K^+ index	Cl^- index	Saluretic Index	Natriuretic Index	Kaliuretic Index	CAI Index
Control	1	1	1	1	1	1	1
Frusemide (5 mg/kg)	1.78	1.97	1.41	1.59	0.89	1.1	0.74
n-hexane (250 mg/kg)	0.8	1.24	0.95	0.88	0.65	1.53	0.94
n-hexane (500 mg/kg)	1.39	1.48	1.07	1.23	0.93	1.07	0.74
Ethyl acetate (250 mg/kg)	1.19	1.56	1.23	1.21	0.76	1.3	0.89
Ethyl acetate (500 mg/kg)	1.97	2.04	1.39	1.68	0.96	1.03	0.69
Water (250 mg/kg)	1	1.32	1.16	1.08	0.75	1.31	1
Water (500 mg/kg)	1.19	1.96	1.25	1.22	0.61	1.64	0.8

Laxative activity Assay

After conducting the laxative test, the reference drug (Bisacodyl 10 mg/kg) increased the stool weight by 80.17% after 16 hours. Similarly, all fractions of F. indica also increased the stool weight. Among these three fractions, the n-Hexane fraction showed the highest increase in stool weight, while the water fraction exhibited the lowest laxative activity (Table 6).

Table 6: Representation of laxative effect of different fractions of *F. indica* extract

Tested group	Dose (mg/kg)	Average weight of stool	% Increase in stool weight
Negative control	------	$0.522 \pm .044^{\theta\,a\,b\,c\,d\,f}$	------
Standard (Bisacodyl)	10	$0.94 \pm 0.04^{*\,a\,b\,c\,d\,e\,f}$	$80.172 \pm 7.721^{*\,a\,b\,c\,d\,e\,f}$
n-Hexane fraction	250	$0.721 \pm 0.056^{*\,\theta\,b\,c\,e\,f}$	$38.22 \pm 10.701^{*\,\theta\,b\,c\,e\,f}$
n-Hexane fraction	500	$0.802 \pm 0.017^{*\,\theta\,a\,c\,d\,e\,f}$	$53.735 \pm 3.386^{*\,\theta\,a\,c\,d\,e\,f}$
Ethyl acetate fraction	250	$0.599 \pm 0.012^{*\,\theta\,a\,b}$	$14.856 \pm 2.302^{*\,\theta\,a\,b}$
Ethyl acetate fraction	500	$0.655 \pm 0.011^{*\,\theta\,b\,e}$	$25.478 \pm 2.167^{*\,\theta\,b\,e}$
Water fraction	250	$0.573 \pm 0.018^{\theta\,a\,b\,d}$	$9.77 \pm 3.522^{*\,a\,b\,d}$
Water fraction	500	$0.623 \pm 0.003^{*\,\theta\,a\,b}$	$19.348 \pm 0.541^{*\,\theta\,a\,b}$

Data are plotted as average of five replicates ± SD (standard deviation);

* $p < 0.05$ vs. Control (Dunnett's t test); $^{\theta}$ $p < 0.05$ vs. Bisacodyl 10 mg/kg; ^{a} $p < 0.05$ vs *n*-Hexane fraction 250 mg/kg; ^{b} $p < 0.05$ vs. *n*-Hexane fraction 500 mg/kg; ^{c} $p < 0.05$ vs. Ethyl Acetate fraction 500 mg/kg; ^{d} $p < 0.05$ vs. Ethyl Acetate fraction 500 mg/kg; ^{e} $p < 0.05$ vs. Water fraction 500 mg/kg; ^{f} $p < 0.05$ vs. Water fraction 250 mg/kg; (pair-wise comparison by Post Hoc Tukey test)

DISCUSSION

The plant kingdom is perhaps one of the most important gifts from nature to humans and other animals. Each plant is a powerhouse of numerous phytochemicals. Phytoconstituents are mainly classified into secondary and primary metabolites. Secondary metabolites are not specifically involved in typical growth and reproduction; instead, they often protect the plant from various diseases. Secondary metabolites are primarily responsible for various biological activities in both the plant and animal kingdoms. Modern medical science heavily relies on various plant-derived drugs. Many important drugs for cardiovascular health, antidiabetic treatment, anticancer therapies, hepatoprotection, antidiarrheal remedies, laxatives, antimicrobial agents, and blood purifiers are directly obtained from various types of natural medicinal plants.[12,13,14] In the primary phytochemical assay of the F. indica extract, Karmakar et al. (2021) reported the presence of saponins, reducing sugars, gums, tannins, flavonoids, steroids, terpenoids, and alkaloids.[2] These phytochemicals might be beneficial for use in various therapeutic purposes, and their bioactivities are already well-documented.

Parasites are organisms that live in or on a living creature of another species (its host) and benefit by stealing vital essential nutrients from the host's body. Many types of parasites live in mammals. In humans, numerous helminths reside in our gastrointestinal tract, where they steal vital nutrients and other essential components from our stomach. As a result, patients suffer from various complications, weight loss, morbidity, etc.[4] These are the causative agents of many diseases such as gastrointestinal upset, dyspepsia, colon and rectal cancer, etc. These problems are mainly found in tropical and subtropical regions worldwide, leading to increased human mortality and causing serious economic losses in the livestock farming business.[15,16,17] High costs, anthelmintic drug resistance, and serious adverse effects have prompted the evaluation of medicinal plants as an alternative source of anthelmintics. Medicinal plants have been used for many decades to treat gastrointestinal helminthiasis, and there is currently a growing demand for plant-derived anthelmintic compounds due to their potency, efficacy, and reduced side effects.[11,5,18] In the anthelmintic assay, we observed that the fractions of F. indica exhibited moderate anthelmintic properties. The different solvent fractions caused both the loosening of motion and death of the P. cervi nematodes in a dose-dependent manner compared to the standard albendazole. Among the three fractions, the n-Hexane fraction showed moderate anthelmintic properties compared to the other two fractions. Therefore, it can be assumed that there may be one or more non-polar anthelmintic compounds present in F. indica. Phytoconstituents in the plant, such as flavonoids, tannins, alkaloids, saponins, and polyphenols, might be responsible for the anthelmintic effect, as their properties have already been reported.[19]

In humans, blood is the most critical fluid that carries vital oxygen and nutrients to the cells while also returning carbon dioxide and harmful substances to be excreted from the body. Blood coagulation (clotting) is the process by which blood changes from a liquid to a gel, thereby stopping bleeding. It is extremely important to stop bleeding in emergencies because excessive blood loss can lead to death. Blood coagulation involves twelve factors that work together to arrest bleeding. The mechanism of coagulation includes the activation, attachment, and accumulation of platelets, along with the deposition and formation of fibrin.[20] Primary hemostasis involves vasoconstriction at the damaged site, preventing blood loss and reducing the diameter of vessels and capillaries. The platelets are activated and then attracted to the site of injury. They also adhere to each other to create a temporary platelet plug over the bleeding area, sealing the injured surface.

On the other hand, secondary hemostasis results in the formation of fibrin over the temporary platelet plug formed during primary hemostasis. This secondary stage involves

the coagulation cascade, which consists of three distinct but interconnected pathways: the intrinsic, extrinsic, and common pathways.[21] So, a decrease in prothrombin time indicates better blood coagulant properties. In the blood coagulation test, we observed that the fractions of F. indica did not exhibit profound coagulant activities. Only the water fraction showed a mild blood coagulant effect. This may be due to the presence of a few polar blood coagulants in the water fraction of F. indica. Phytoconstituents such as saponins, tannins, flavonoids, and steroids might be responsible for this blood coagulant effect, as they are well-documented for having this property.[22]

Constipation is a very common gastrointestinal disorder in which stool becomes dry, hard, and difficult to expel. Other symptoms may include painful spasmodic bowel movements, dyspepsia, acute stomach pain, and discomfort, among others. These factors can contribute to various colorectal problems such as hemorrhoids and colorectal malignancy. Globally, constipation affects 8-15% of the population. However, constipation is often a result of various underlying issues rather than a single disorder on its own. Potential causes include digestive disorders, metabolic and endocrine disorders, and neurological problems. Additionally, many medications can produce constipation as a side effect, including anticholinergics, antidepressants, iron supplements, and aluminum-containing compounds.[23] In the treatment of constipation, plant-derived laxatives have been used for centuries. Laxatives can work by retaining water inside the bowel lumen through osmotic effects or by stimulating intestinal secretion or motility, which increases the amount of intestinal bulk. This, in turn, increases stool weight and makes the stool softer and easier to expel. However, due to a lack of efficacy, the treatment of constipation with available drugs is often insufficient for reducing bloating and other associated symptoms.[23] Acetylcholine plays a significant role as the principal excitatory neurotransmitter in the enteric neurological network. Hence, the existence of cholinomimetic components within the plant may clarify the value of the laxative effect. Besides, previous experiments have revealed that the laxative properties of plants depend on the presence of phytochemicals like flavonoids, tannins, terpenoids, alkaloids, sterols, and phenolic compounds.[11,24]

In the laxative test, we found that the F. indica n-Hexane fraction exhibited the best laxative effect. At doses of 250 and 500 mg/kg, the stool weight increased by up to 38% and 54%, respectively, while bisacodyl increased the stool weight by 80% at a dose of 10 mg/kg. However, the other two fractions did not show such an increase in stool weight as the n-hexane fraction. It is already reported that phytoconstituents such as saponins, flavonoids, tannins, sterols, alkaloids, terpenoids, and phenolic components in various fractions of plant extracts are found to be responsible for laxative, stimulant, and alimentary peristaltic properties.[11,24] So, we may assume that this F. indica plant might also possess these types of compounds, especially in the n-Hexane fraction.

Hypertension, the elevation of blood pressure from normal values, is considered one of the dominant health problems and a significant well-being challenge worldwide. The World Health Organization (WHO) reports that one out of every eight deaths is caused by hypertension globally. In a recent survey, it was observed that approximately one-third of the adult world population is suffering from hypertension (31.1%, 1.39 billion), and this issue is more prominent in low- and middle-income countries. Chronic smoking, alcohol consumption, a diet rich in fat, obesity, mental stress, sedentary lifestyle, hypercholesterolemia, and hyperglycemia are the main reasons for hypertension. Hypertension also contributes to the development of other serious cardiovascular diseases.[25]

Diuretics are the first-line drugs used to treat hypertension and related complications. Different types of diuretics work by reducing the reabsorption of water and electrolytes in the nephrons. They are medically significant in the treatment of clinical conditions such as nephrotic problems, hypertension, and cardiovascular disorders.[9]

Various medicinal plants are traditionally used to treat hypertensive complications because they possess numerous secondary metabolites that are useful in this regard. In the diuretic test, we observed that different fractions of F. indica leaves increased urinary output as well as diuretic activity over time. These observations indicated diuretic-like effects in the mice, which were further confirmed by electrolyte analysis. Controlling the levels of K+, Na+, and Cl- in the blood is exceptionally significant for regulating blood volume, blood pressure, cardiac output, acid-base balance, and maintaining the functionality of heart muscles. In our test, we used frusemide as the standard drug. Frusemide is a recognized loop diuretic that acts in the ascending part of the loop of Henle. It inhibits sodium reabsorption, leading to significant urinary sodium and chloride excretion.[26] After conducting the diuretic test, we also performed an electrolyte analysis of the urine. Consequently, we calculated the natriuretic activity, kaliuretic activity, and saluretic activity for different fractions of F. indica. Subsequently, we measured CAI activity. In our diuretic and urinary electrolyte analysis test, we found that the F. indica ethyl acetate fraction at 500 mg/kg doses exhibited the best diuretic activity, and a diuretic index exceeding 1.5 indicates a good diuretic effect.[27] On the other hand, the ethyl acetate fraction at 500 mg/kg doses also exhibited the best Na+, K+, Cl-, saluretic, and natriuretic indexes. The carbonic anhydrase enzyme is responsible for the production of H_2CO_3 and ultimately HCO_3- in the nephron. Because of this enzymatic activity, HCO_3- ions can be readily reabsorbed from proximal tubules. So, the inhibition of carbonic anhydrase may indicate good diuretic properties, and a CAI (carbonic anhydrase inhibition) index lower than 0.8 indicates the best diuretic effect.[9] Based on this information, once again, the ethyl acetate fraction of F. indica extract at a dose of 500 mg/kg showed the best diuretic effect. Considering these overall diuretic effects, we can assume that the ethyl acetate fraction of F. indica contains both loop diuretics and carbonic anhydrase inhibitors. Phytochemicals like flavonoids, organic acids, saponins, polyphenolic compounds, alkaloids, and steroids might be responsible for this diuretic effect, as their diuretic activity has already been reported.[28]

CONCLUSION

Medicinal plants have been used for centuries for numerous therapeutic purposes. From our observations, we have found that different fractions of F. indica leaves can be used as anthelmintic, blood coagulant, diuretic, and laxative agents. These preliminary results might be helpful for natural product researchers to isolate pure bioactive compound(s) from this plant in the future, and this could lead to the development of new drugs derived from this plant.

Data availability

All reported data are preserved by the authors and will be made accessible upon request for clarification.

Conflict of interest

The authors hereby declare that there is no conflict of interest among them, and they were all informed about this matter before submitting this article.

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استكشاف الأنشطة المضادة للديدان، ومخثر الدم، ومدر للبول، وملين لأجزاء المذيبات المختلفة لأوراق فلاجيلاريا إنديكا

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ملخص

فلاجيلاريا إنديكا (الأسرة: فلاجيلارياسيا) نبات قشور شائع يوجد بشكل رئيسي في المناطق الاستوائية في العديد من البلدان. للنبات بعض الاستخدامات التقليدية ولكن معظمها لا يحتوي على تقارير علمية منشورة. تم تجزئة المستخلص الإيثانولي الخام لأوراق F. Indica اعتمادًا على القطبية باستخدام الماء، وخلات الإيثيل، و n-Hexane. تم إجراء فحص بيولوجي للأنشطة المضادة للديدان، وتخثر الدم، ومدر للبول، وملين باستخدام الماء، وخلات الإيثيل، و n-Hexane من أوراق F. في اختبار طارد الديدان، أظهر جزء n-Hexane تأثيرًا معتدلًا مع فترات الشلل كانت 16.79 و 13.62 دقيقة وأوقات الوفاة كانت 27.34 و 21.81 دقيقة على التوالي بجرعة 25 و 50 مجم / مل. في اختبار تخثر الدم، أظهر جزء الماء تأثيرًا ملحوظًا (كانت أوقات التخثر 4.33 و 6.02 و 7.68 و 8.32 دقيقة على التوالي بجرعة 200 و 100 و 50 و 25 مجم / مل). تم إجراء زيادة حجم البول المفرز وتحليل الكهارل في البول لتحديد الرقم الهيدروجيني والكثافة والتوصيل و Na + وCl- وK + وnatriuretic و kaliuretic saluretic و CAI index. أظهر جزء أسيتات الإيثيل نشاط مدر للبول أفضل من جزء الهكسان n بينما لم يكشف جزء الماء عن تأثير مدر للبول ملحوظ. تم العثور على فهارس Na +وCl- وK + وnatriuretic و saluretic مرضية في جزء خلات الإيثيل وتم العثور على مؤشر CAI بشكل أفضل في جزء n-Hexane. في اختبار الملين، أظهر جزء n-Hexane أفضل خصائص ملين بينما تم العثور على الزيادة في وزن البراز بنسبة 38٪ و 54٪ عند جرعات 250 و 500 مجم / كجم على التوالي. تشير هذه النتائج إلى أن الأجزاء المختلفة من أوراق F. Indica لها مواد كيميائية نباتية مميزة قد تكون مسؤولة عن مثل هذه التأثيرات البيولوجية. قد يكون عزل المركبات النشطة بيولوجيًا مبررًا لاستخداماته التقليدية في الطب الحديث.

الكلمات الدالة: فلاجيلاريا إنديكا، طارد للديدان، مخثر الدم، مدر للبول وأنشطة ملين.

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C-Jun N-Terminal Kinases Inhibition: A New Approach to the Regulation of Venlafaxine Pharmacokinetics

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ABSTRACT

The effect of the c-Jun N-terminal kinases (JNK) inhibitor 'IQ-1' on the pharmacokinetics of the antidepressant venlafaxine was studied. An acceleration of the metabolism of this psychotropic agent was revealed when a modifier of intracellular signal transduction was administered to experimental animals in vivo. The JNK blockade was accompanied by a decrease in the plasma level of the antidepressant without changes in the concentration of the pharmacologically active metabolite O-desmethylvenlafaxine. The results obtained indicate a modification of the pattern of venlafaxine biotransformation, involving a change in metabolic pathways with an increase in the formation of other metabolites, or a correction of its distribution in the body. The revealed properties of the JNK inhibitor can be used to develop fundamentally new approaches to improve the effectiveness of antidepressant therapy with venlafaxine within the framework of implementing the 'Strategy for Targeted Regulation of Xenobiotic Metabolism and Drug Pharmacokinetics'.

Keywords: Metabolism, Pharmacokinetics, Antidepressant Therapy, Intracellular Signal Transduction, JNK.

INTRODUCTION

The functioning of all cell types relies on the participation of the intracellular signal transduction system [1-6]. However, the specific roles of individual signal transduction pathways in regulating the xenobiotic-metabolizing function of cells competent in this regard are largely unknown. Simultaneously, uncovering the involvement and distinct roles of particular signaling molecules in the biotransformation of pharmacologically active substances can form the basis for the development of innovative approaches to personalized pharmacotherapy [7-11]. Therefore, it is pertinent to investigate the potential for controlling the intensity and nature of substance transformation within the body by regulating intracellular signal transduction in metabolizing cells and creating 'Targeted Regulators of Xenobiotic/Drug Metabolism' [7, 12].

In recent years, pharmacologists have increasingly focused on modifiers affecting the activity of mitogen-activated protein kinase known as c-Jun N-terminal kinase (JNK). It has been demonstrated that JNK plays a role in regulating various functions of different cellular components, and inhibitors targeting this signaling molecule have been identified to possess neuroprotective, anti-inflammatory, hemostimulatory, and several other pharmacological properties [2]. Additionally, the significant role of JNK in regulating the metabolism of the antidepressant venlafaxine by liver cells is well-documented [7, 12].

Venlafaxine, a widely used antidepressant, exists as a

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racemate of R- and S-enantiomers, effectively blocking the reuptake of serotonin, norepinephrine, and dopamine [13, 14]. However, its effectiveness is sometimes compromised due to variations and individual characteristics in the pathogenesis of depressive disorders. Venlafaxine is primarily metabolized in liver cells with the assistance of cytochrome P450 enzymes [9, 15]. The CYP2D6 isoenzyme, in particular, converts it into the sole pharmacologically active metabolite, O-desmethylvenlafaxine (O-DVLF) [13, 16]. Additionally, CYP2C19, CYP3A4, and CYP2C9 contribute to the formation of inactive metabolites, including N, O-didesmethylvenlafaxine, and their glucuronide conjugates [9, 17]. Developing specific methods to modify the pharmacokinetics and pharmacodynamics of this drug holds the potential to significantly enhance the quality of psychiatric care for patients with depression.

We have previously demonstrated the potential for accelerating the conversion of venlafaxine to O-DVLF using a JNK inhibitor in vitro [7, 12]. However, the precise nature of the modification of this antidepressant's metabolism with selective JNK blockade in vivo and the resulting alterations in its pharmacokinetics remain unknown.

The objective of this study was to investigate the impact of a JNK inhibitor on the pharmacokinetic profiles of venlafaxine and O-desmethylvenlafaxine in blood plasma.

MATERIALS AND METHODS

Animals and experimental design

The study was conducted on 114 C57BL/6 mice aged 2-2.5 months, weighing 20-22 g, at E.D. Gol'dberg Tomsk NIMC. All experimental procedures followed ethical principles for the humane treatment of animals and received approval from the local Ethics Committee of the Goldberg Research Institute of Pharmacology and Regenerative Medicine, Tomsk National Research Medical Center, Russian Academy of Sciences (protocol GRIPh & RM-2022-01/12).

The aimals were randomly assigned to two groups: control and experimental, with 48 mice in each group. The experimental group received venlafaxine (supplied by Aarti Industries, India) via intragastric tube at a dose of 120 mg/kg (2.4 mg/mouse), selected based on literature data from a pharmacokinetic study of venlafaxine in small rodents. Fifteen minutes before venlafaxine administration, the experimental group of mice was intraperitoneally injected with the JNK inhibitor "IQ-1S" (11H-indeno[1,2-b]quinoxalin-11-one oxime sodium salt, from Montana State University, Bozeman, Montana, USA) at a dose of 30 mg/kg (0.6 mg/mouse), a dose commonly used in the study of the pharmacological properties of "IQ-1S" in mice. The control group received an equivalent volume (0.2 ml) of solvent intraperitoneally.

Blood samples were collected at 0.25, 0.5, 1, 2, 3, 4, 8, and 24 hours (n=6 for each observation period in both control and experimental groups) after the administration of venlafaxine. The blood was obtained from the heart under deep anesthesia in a CO2 chamber, in a volume of 0.5 ml. Plasma was then obtained by centrifugation at 1660×g for 10 minutes, and the levels of venlafaxine (VLF) and O-desmethylvenlafaxine (O-DVLF) were determined [13, 17].

VLF and O-DVLF definition

The sample preparation method was based on the principle of liquid-liquid extraction using an organic solvent. Initially, 200 µl of thawed plasma was combined with 100 µl of an internal standard solution, which contained fluvoxamine hydrochloride at a concentration of [C] = 50 ng/ml. To this mixture, 50 µl of 12.5% ammonia solution, 300 µl of 0.9% sodium chloride solution, and 800 µl of ethyl acetate were added. The resulting mixture was then vigorously stirred using an MSV-3500 tube vortexer (Biosan, Latvia) at 2100 rpm for 8 minutes. Subsequently, the phases were separated by centrifugation in an SL 16L centrifuge (Thermo Scientific, USA) at 12000×g for 8 minutes at 4°C. The upper organic fraction, comprising

650 µl, was carefully transferred into a colorless glass vial for subsequent chromatographic and mass spectrometric analysis.

The quantitative determination of VLF and O-DVLF in mouse plasma was conducted using HPLC-MS/MS, employing an LC-20 Prominence liquid chromatograph from Shimadzu in conjunction with an AB Sciex QTrap 3200 tandem mass spectrometer featuring electrospray ionization.

The equipment setup included an LC-20AD high-pressure mobile phase pump, SIL-20A auto-injector, and CTO-20A column thermostat. The analytical column used was a Phenomenex Luna C18, with a particle size of 5 µm, measuring 100 × 4.6 mm, and it was accompanied by a pre-column cartridge. The mass spectrometry analysis was performed using the AB Sciex QTrap 3200 tandem chromatograph-mass spectrometer. Data acquisition was carried out with Analyst 1.6.3 software, while chromatographic data processing was accomplished using Multi Quant 2.1.

The fragmentation and detection of analytes were achieved through electrospray ionization in the specified reaction monitoring mode, where positively charged ions were recorded. This process was based on the following transitions, measured as m/z (mass-to-charge ratio): for VLF, 278.0 (parent ion) to 58.0 (VLF fragment ion), and for O-DVLF, 264.1 (parent ion) to 57.90 (fragment ion).

Chromatographic analysis was conducted in an isocratic mode, utilizing a mobile phase composed of acetonitrile (eluent B) and 5 mM aqueous ammonium formate, pH 2.93 (eluent A), at a ratio of 85:15 (v/v) and a flow rate of 0.65 ml/min. A 2 µl aliquot was injected, and the column was maintained at a constant temperature of 40°C. The average retention time for VLF and O-DVLF was 1.49±0.01 and 1.44±0.02 minutes, respectively, and the entire analysis process took no longer than 3.50 minutes. As an internal standard, fluvoxamine (m/z, 319.1 → 71.1) was employed, with an average retention time of 1.50±0.02 minutes.

Calibration curves were constructed by plotting the ratio of analyte peak area to the internal standard peak area against the nominal sample concentration, using a weighted least squares method with a weighting factor of 1/x^2, where x represents the nominal analyte concentration. The calibration curve range was 0.5 to 105,000 ng/ml for VLF (n = 6, y = 0.0132x + 0.0100, R^2 = 0.9937) and 0.5 to 500 ng/ml for O-DVLF (n = 6, y = 0.0130x + 0.0101, R^2 = 0.9930). A total of 18 mice were used for method validation and calibration curve construction

The extramodel method of statistical moments was calculated using Phoenix Phoenix WinNonlin® version 8.3 (Certara, USA): time to reach maximum concentration (Tmax, h), maximum plasma concentration (Cmax, ng/ml), area under the plasma concentration-time curve from the moment of taking the drug to 24 h (AUC 0-24, h×ng/mL), area under the plasma concentration-time curve from the moment of drug administration to infinity (AUC 0-∞, h×ng/mL), mean residence time of the compound in the systemic circulation (MRT, h), half-life (T½, h), apparent volume of distribution (Vd/F, ml), total clearance (plasma volume that is completely cleared of the drug per unit time, Cl/F, ml/h).

Statistical analysis

The results obtained were analyzed using the method of variance statistics with the STATISTICA 6.0 analysis package. To compare all pharmacokinetic parameters, except for Tmax, the Student's t-test was applied after logarithmic data transformation. For Tmax, the nonparametric Mann–Whitney U test was used. The values are presented as the arithmetic mean and standard error of the mean (M±SEM), and the significance of differences in indicators between groups was considered at $p<0.05$.

RESULTS AND DISCUSSION

Pharmacokinetics of venlaflaxine

Following a single intragastric administration of venlafaxine to mice, rapid absorption into the bloodstream occurred, with peak concentrations reached at 0.25, 0.5, and 1 hour of observation (Table 1, Fig. 1). The time to

reach maximum concentration was 0.5 hours (Table 2). Subsequently, there was a notable decline in circulating VLF levels, reaching their lowest point at the 24-hour mark in the experiment. These results align closely with the pharmacokinetic characteristics of the studied antidepressant, as reported in.

Table 1: Dynamics of the concentration of venlafaxine (VLF) and its metabolite O-desmethylvenlafaxine (O-DVLF) in the blood plasma of C57BL/6 mice after the single administration of venlafaxine (1) and with the combined use of an antidepressant with the JNK inhibitor (2), ng/ml (M±SEM)

Time, h	VLF		O-DVLF	
	1	2	1	2
0.25	3521.45±562.38	2539.72±416.43	473.03±12.38	464.89±19.44
0.5	3962.16±795.01	2937.10±622.79	395.17±31.43	363.99±43.76
1	3863.39±297.82	2713.83±189.37*	244.08±20.93	257.63±15.42
2	1067.61±98.21	613.98±55.08*	99.94±11.20	85.51±7.67
3	1116.86±100.18	1539.15±210.43	62.48±5.40	61.45±12.52
4	710.37±41.2	511.27±49.68*	71.94±7.03	61.92±8.70
8	62.93±10.6	63.58±11.64	13.03±2.17	8.64±2.31
24	5.11±1.25	8.91±1.41	0.73±0.13	0.99±0.43

*** - the differences in the indicator with the control (Group1) at p<0.05**

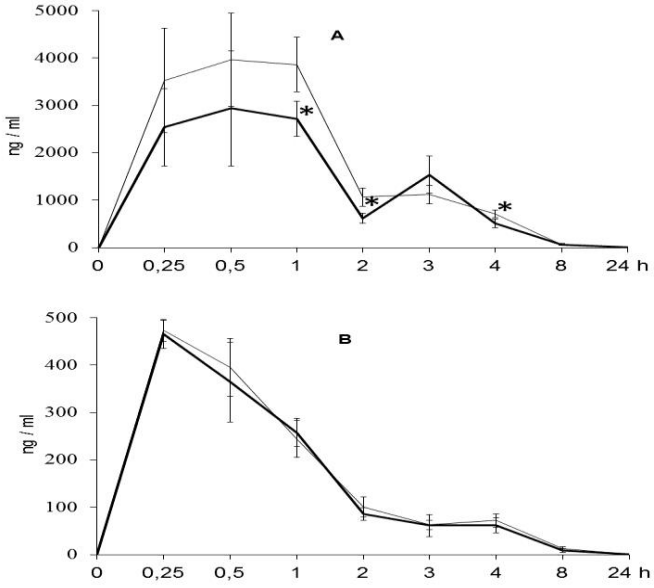

Fig. 1. Mean pharmacokinetic profiles of venlaflaxine (A) and its metabolite O-desmethylvenlafaxine (B) in blood plasma of C57BL/6 mice after a single administration of venlaflaxine (thin lines) and the antidepressant with the JNK inhibitor (thick lines). Confidence intervals at p=0.05; * - the significance of differences in indicators with control was noted at p<0.05.

Prior oral administration of the JNK inhibitor significantly influenced the studied pharmacokinetic parameters. In the initial phase of the study, the plasma concentration of VLF remained comparable to control values, indirectly suggesting a limited impact of the JNK activity modifier on the drug absorption process. However, at 1, 2, and 4 hours of observation, there was a statistically significant reduction in the VLF concentration in plasma, reaching levels of approximately 70.2%, 57.5%, and 71.9% of the corresponding control values, respectively (Table 1, Fig. 1).

Table 2: Pharmacokinetic parameters of venlaflaxine (VLF) and its metabolite O-desmethylvenlafaxine (O-DVLF) following the single administration of venlaflaxine (1) and co-administration of the antidepressant with the JNK inhibitor (2) in C57BL/6 mice, (M±SEM)

Parameters	VLF		O-DVLF	
	1	2	1	2
Tmax (h)	0.70±0.12	1.15±0.29	0.30±0,05	0.25±0.00
Cmax (ng/ml)	4283.19±522.55	3730.45±401.12	476.08±12.41	464.89±19.44
AUC 0-24 (h×ng/ml)	9700.97±490.46	8035.53±485.71*	927.91±47.55	842.08±49.82
AUC 0-∞ (h×ng/ml)	9724.39±494.94	8082.55±490.34*	931.26±47.82	847.52±49.92
MRT (h)	2.27±0.14	2.49±0.12	2.79±0.15	2.54±0.25
T 1/2 (h)	2.86±0.12	3.45±0.16*	3.12±0.11	3.27±0.32
Vd/F (ml)	1026.59±37.74	1557.42±131.37*	-	-
Cl/F (ml/h)	256.61±14.68	287.22±26.63	-	-

*** The differences in the indicator with the control (Group1) at $p<0.05$**

The analysis of the calculated pharmacokinetic parameters in this case indicated a decrease in both AUC 0-24 and AUC 0-∞ (reducing to 82.2% and 83.1% of the control levels, respectively) (Table 2). Simultaneously, there was an increase in the half-life of VLF (by 20.1% from the initial values) and a notable expansion in the volume of distribution of the agent, reaching 151.7% of the control level. The estimated total clearance of VLF did not show significant differences between the control and experimental groups.

The examination of the content of the primary metabolite of the antidepressant in blood plasma also revealed interesting phenomena concerning the drug's targeted effect on its pharmacokinetics. Specifically, Tmax for O-DVLF was just 0.5 hours, with MRT at 2.79 hours and a half-life of 3.12 hours (Table 2, Fig. 1). These characteristics align with literature data concerning the rapid first-pass metabolism of venlafaxine, resulting in the formation of the active metabolite O-desmethylvenlafaxine [16, 17].

The administration of the JNK inhibitor before venlafaxine did not lead to statistically significant changes in the O-DVLF concentration throughout the entire observation period (Table 1, Fig. 1). No corrections in the calculated parameters were observed. It's important to note that dose-dependent parameters such as Cl/F (clearance) and Vd/F (apparent volume of distribution) are not typically calculated for metabolites. However, in this context, it seems reasonable to anticipate an accelerated uptake into tissues under the influence of the JNK inhibitor, not only for VLF (as suggested earlier) but also for O-DVLF. This is because an increase in the intensity of venlafaxine biotransformation into O-DVLF under the influence of a JNK inhibitor [7, 12] should logically result in an increased plasma concentration of this metabolite.

Overall, the results obtained indicate a significant alteration in the pharmacokinetics of venlafaxine when influenced by the JNK inhibitor. The observed decrease in plasma concentration of VLF, while O-DVLF remains present, may be associated with several mechanisms.

First, it could be linked to the previously identified acceleration and alteration in the metabolic pathways of venlafaxine, resulting in a new metabolic pattern, particularly an increase in the formation of N, O-didesmethylvenlafaxine through the activation of CYP2C19, CYP3A4, and CYP2C9 [7, 12].

Secondly, this phenomenon could be rooted in redistribution mechanisms affecting both VLF and O-DVLF, involving their rapid penetration into tissues. It's likely that the observed increase in VLF's half-life (T½) is associated with this process. With a higher substance intake into tissues, less of it can be immediately excreted from the body, as there is a reduced amount of VLF circulating in the bloodstream at any given time. The removal of VLF becomes possible only when the substance re-enters the bloodstream from the tissues.

Moreover, the confirmation of the involvement of this mechanism (rapid tissue penetration of VLF) in the observed patterns could serve as a foundation for developing a fundamentally new approach to enhancing the efficacy of antidepressant therapy with venlafaxine. Given the relatively high affinity of both VLF and O-DVLF for nervous tissue [8], this opens up prospects for increasing the concentration of both venlafaxine itself (which possesses independent psychotropic pharmacological activity [13]) and its active metabolite (O-DVLF) [7, 17] in the brain. This is particularly significant considering their relatively high affinity for nervous tissue, especially venlafaxine [18].

As a result, there is the potential not only to enhance therapeutic efficacy but also to reduce the overall xenobiotic burden on the body, consequently lowering the risk of drug-related side effects, all while maintaining therapeutic effectiveness.

CONCLUSION

The findings indicate the need for further investigation into the potential of JNK inhibitors to modify the pharmacokinetics of venlafaxine, as part of the development of the 'Strategy for Targeted Regulation of Xenobiotic Metabolism and Drug Pharmacokinetics' [7, 12].

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مثبط JNK: نهج جديد لتنظيم الحرائك الدوائية للفينلافاكسين

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ملخص

تمت دراسة تأثير مثبط "IQ-1" JNKعلى الحرائك الدوائية لمضاد الاكتئاب فينلافاكسين. تم الكشف عن تسارع عملية التمثيل الغذائي لهذا العامل المؤثر عقليًا عندما تم إعطاء معدل نقل الإشارة داخل الخلايا لحيوانات التجارب في الجسم الحي. ترافق حصار JNK مع انخفاض في مستوى البلازما لمضاد الاكتئاب دون تغييرات في تركيز المستقلب النشط دوائيًا .O-desmethylvenlafaxine تشير النتائج التي تم الحصول عليها إلى تعديل نمط التحول الحيوي للفينلافاكسين (والذي يتكون من تغيير في المسارات الأيضية مع زيادة تكوين المستقلبات الأخرى) أو تصحيح توزيعه في الجسم. يمكن استخدام الخصائص التي تم الكشف عنها لمثبط JNK لتطوير مناهج جديدة بشكل أساسي لتحسين فعالية العلاج المضاد للاكتئاب باستخدام فينلافاكسين في إطار تنفيذ "استراتيجية التنظيم المستهدف لعملية التمثيل الغذائي للأجسام الحيوية وحركية الدواء".

الكلمات الدالة: التمثيل الغذائي، الحرائك الدوائية، العلاج بمضادات الاكتئاب، نقل الإشارات داخل الخلايا، JNK.

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المجلة الأردنية في العلوم الصيدلانية

تعريف بالمجلة الأردنية في العلوم الصيدلانية

تأسست المجلة الأردنية في العلوم الصيدلانية بقرار لجنة البحث العلمي/ وزارة التعليم العالي والبحث العلمي رقم 367/2/10 تاريخ 2007/1/11 بشأن إصدار "**المجلة الأردنية في العلوم الصيدلانية**" ضمن إصدارات المجلات الأردنية الوطنية، وهي مجلة علمية عالمية متخصصة ومحكمة، وتصدر بدعم من صندوق دعم البحث العلمي والجامعة الأردنية تعنى بنشر البحوث العلمية الأصيلة المقدمة إليها للنشر في كافة مجالات العلوم الصيدلانية والعلوم الأخرى المرتبطة بها. وتصدر عن عمادة البحث العلمي وضمان الجودة في الجامعة الأردنية باسم الجامعات الأردنية كافة، خدمة للمتخصصين والباحثين والمهتمين في هذه المجالات من داخل الأردن وخارجه. وهي مجلة تصدر أربع مرات في العام أعتبارا من 2021، ومواعيد صدورها (آذار وحزيران وأيلول وكانون أول) من كل عام.

وباسمي وباسم أعضاء هيئة التحرير نود أن نشكر الزملاء الذين أسهموا بإرسال أبحاثهم إلى مجلتنا وتمكنا من إخراج العدد الأول. ونأمل من جميع الزملاء بإرسال ملاحظاتهم الإيجابية إلينا لنتمكن من النهوض بمجلتكم بالشكل الذي يليق بها.

وهذه دعوة إلى كافة الزملاء لإرسال اسهاماتهم العلمية من الأبحاث الأصيلة إلى عنوان المجلة.

والله ولي التوفيق

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