Evaluation of the Therapeutic Effect of Cardamom Extract on Nephropathy Induced by Aspirin in Rats Model

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ABSTRACT

Analgesic-induced nephropathy is a serious complication resulting from the chronic overuse of analgesics, especially over-the-counter drugs such as aspirin. The study aimed to investigate the therapeutic effect of cardamom extract on aspirin-induced nephropathy in rat models. Twenty-four female Albino Wistar rats were randomly divided into three groups (n=8/group): control(no treatment); (ASA) aspirin 600 mg/kg/day for 4 days, and (ASA + Card) aspirin + cardamom extract 200 mg/kg/day for 7 days. Cardamom aqueous extract was prepared. Phenol and flavonoid contents were calculated. The kidney/body weight ratio was calculated, and serum urea and creatinine were measured. Lipid peroxidation was evaluated by measuring Malondialdehyde (MDA) concentrations, and superoxide dismutase (SOD) activity in kidney tissue. Histological alterations were also assessed. Parametric data were evaluated using the One-way analysis of variance (ANOVA) test, followed by Tukey's test. Nonparametric data were evaluated by the Mann–Whitney test and Fisher's tests. The results were considered significant at $P<0.05$. Total phenol 23.4 mg gallic acid equivalents / g dry extract, and flavonoids 1.77 mg quercetin equivalents /g dry extract. In the ASA group, kidney weight/body weight ratio, Serum biomarkers, and MDA concentrations were significantly increased, while SOD levels decreased, compared with the control group. The histological examinations showed significant tubular and glomerular injuries. There was a significant improvement in the Card histological and serum when compared with the aspirin group. Cardamom aqueous extract (200 mg/kg) showed effective therapeutic ability against aspirin-induced nephropathy by improving kidney functions, and enzymatic and histological parameters, due to their antioxidant activity in oxidative stress induced by aspirin.

Keywords: Kidney; Nephropathy; Nonsteroidal Anti-Inflammatory Drugs; Aspirin; Cardamom Extract; Oxidative Stress.

INTRODUCTION

Aspirin (acetylsalicylic acid) is a nonsteroidal anti-inflammatory drug (NSAID) that has antiplatelet, anti-inflammatory, antipyretic, and analgesic effects in the treatment of rheumatoid arthritis and prevention of thrombotic cardiovascular disease [1]. Long-term therapeutic administration of aspirin is associated with its harmful effects on the kidney, which can lead to renal disorders. Aspirin also initiates oxidative stress in the kidney by inhibiting the activity of antioxidant enzymes in mitochondria [2]. It also increases the production of reactive oxygen species (ROS), increasing the possibility of apoptosis and necrosis within the kidney cells [3], and that ends with many pathological effects such as tubular necrosis and tubulointerstitial nephritis [4]. Recently,

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interest has increased in natural antioxidants from medicinal plant sources as a more acceptable and safe choice than chemical ones [5]. The previous studies focused on plants that have high contents of polyphenols (phenols and flavonoids), which are secondary metabolites with significant antioxidant activity [6,7]. It is essential to focus on therapeutic options for oxidative stress-induced diseases that have not been commonly used before, such as cardamom, one of the most widely available and tolerable medicinal plants.

Cardamom (Elettaria cardamomum (L.) Maton) is a perennial herbaceous plant belonging to the Zingiberaceae family and grows in India, Guatemala, Sri Lanka, Nepal, and Indonesia. It is the third most expensive spice after saffron and vanilla. Cardamom is a traditional medicine used for several diseases like flatulence, bronchitis, asthma, kidney disorders, arthritis, congestion, and itching. Cardamom is a rich source of antioxidant components of phenolic compounds like quercetin, kaempferol, and luteolin that can suppress free radicals activity by preventing oxidation reactions, thus leading to reducing cellular injury [8]. Previous studies investigated the antioxidant properties of cardamom extracts either through protective effects in various oxidative stress-related diseases [9] or through therapeutic effects [10]. Cardamom almost reverses all the mechanisms by scavenging the formation of free radicals due to its high contents of flavonoids [11].

This study investigates the possible therapeutic effects of aqueous cardamom in preventing aspirin-induced nephropathy in rats.

MATERIALS AND METHODS

2.1 Animals

Twenty-four adult female Wister albino rats weighing (100-200) g were obtained from the animal breeding colony of the Atomic Energy Commission of Syria, Damascus, Syria. The rats were maintained in clean plastic cages and provided ad libitum and water. The laboratory animal room was under controlled environmental conditions (temperature 23±2°C, humidity 55±15%, 12-hour light/dark cycle), and the animals were acclimatized for one week before the start of experiments. All procedures in this study conformed to the guiding principles for research-involving animals and were approved by the Research Ethical Committee of the Faculty of Pharmacy, Damascus University, and conducted according to the guidelines of the National Institutes of Health (NIH) for the Care and Use of Laboratory Animals.

2.2 Experimental design

The rats were randomly divided into three groups (8 rats in each group):(Control): no treatment; (ASA): aspirin-CMC suspension (600 mg/kg/day P.O) at 24 h intervals for four days [12]. (ASA + Card): Cardamom aqueous extract (200 mg/kg P.O) beginning 24 h after the fourth aspirin dose at 24 h intervals for seven days [13]. At the end of the experimental period, the animals from each group were sacrificed. Blood samples were collected through heart puncture and serum was separated for renal function tests (BUN and creatinine). The kidneys were excised and weighed, and the macroscopic change was checked. One kidney was stored at −80°C and then homogenized for oxidative stress biochemical analysis (MDA, SOD), while the other one was sectioned longitudinally into two equally sized pieces and fixed in a 15% buffered formalin solution for histopathological studies.

2.3 Drugs

Aspirin (ASA): (The global company for pharmaceutical industries, Unipharma).

2.4 Plant material

The dried Cardamom fruits (E. cardmomum) were obtained from Al-Attar Herbal Pharmaceutical Industries (Damascus, Syria) and ground into a fine powder to make the aqueous extract.

2.5 Preparation of aqueous extract

Twenty grams of cardamom fine powder were added

to 100 ml distilled water, mixed well, and then kept at room temperature for 24 h on a magnetic stirrer with 2500 rpm. The mixture was filtered using filter paper. The resulting filtrate was centrifuged at 5000 rpm for 15 min, and the supernatant was filtered. The concentration of the filtrate (aqueous extract) was adjusted with distilled water to reach a final concentration of 200 mg/mL and collected in sterilized test tubes cotton plugged and stored in a refrigerator until ready to use [14,15]. The extract showed an extractive yield (7%).

2.5.1 Total phenol content determination

The total phenol contents of aqueous extracts were determined based on the Folin-Ciocalteu method described by [16], with some modulations. 20 µL of the aqueous extract was mixed with 1.58 ml of distilled water and then added to 100 µL of Folin-Ciocalteu reagent. 300 µL of 20% sodium carbonate was added after 5 minutes. The resulting greenish-blue solution was incubated in a dark place at room temperature for 2 hours. The absorbance was measured at 765 nm. Gallic acid was used as a calibration curve standard, using five serial dilutions (0, 100, 200, 250, 300, 400, and 500 mg/L) in methanol, which was used as a blank, and each reading was triplicated. The total phenol content was expressed as milligrams of gallic acid equivalent (GAE) per g of extract.

2.5.1 Total flavonoid content measurement

Total flavonoids in the aqueous extracts were determined by the aluminum chloride colorimetric method as mentioned by [17] with few modifications. 2 ml of aqueous extract mixed with 0.1 mL aluminum chloride (10%) and 0.1mL sodium acetate (1M) and then with 2.8 ml of distilled water. The absorbance was taken at 415 nm after incubation for 30 min at room temperature. Quercetin was used as a standard to construct the calibration curve using six different concentrations (0, 2.5, 5, 10, 15, 20µg/mL) in methanol solvent. The estimation of total flavonoids in the extracts was triplicated, and the results were averaged. The concentrations were expressed as milligrams of quercetin equivalent per g of extract.

2.6 Kidney weight/body weight ratio (%)

The rats were checked for food intake and body weight at regular time intervals from day 0 to day 11 to calculate the body weight change ratio (%). Kidney weight/ body weight change ratio (%) was also calculated.

2.7 Serum Creatinine Concentration

Serum creatinine levels were estimated by an assay Kit (Biosystems Company). The creatinine of the sample reacts with picrate in an alkaline medium and forms a colored complex that was measured spectrophotometrically at 500 nm, and the concentrations were calculated accordingly.

2.8 Serum Urea Concentration

Serum urea levels were estimated by an assay Kit (Urea/BUN- Biosystems). The urea of the sample reacts with the kit's reagent components (urease, nitroprusside, salicylate, and NaClO), which forms a colored complex that was measured spectrophotometrically at 600 nm, and the concentrations were calculated accordingly.

2.9 Determination of oxidative stress biomarkers

2.9.1 lipid peroxidation

Lipid peroxidation (LPO) is a kidney injury indicator induced by reactive oxygen species (Kongkham et al., 2013). Malondialdehyde (MDA), as a marker for LPO, was determined by measuring thiobarbituric acid reactive substance (TBARS). Briefly, 0.5 ml of kidney tissue homogenates prepared were reacted with 2 ml of TBA reagent (0.375% TBA, 15% trichloroacetic acid, and 0.25 N HCl). Samples were boiled for 15 minutes, cooled, and centrifuged. The absorbance of the supernatants was measured spectrophotometrically at 532 nm. The concentration of MDA was calculated by the absorbance coefficient of the MDA–TBA complex (1.56 × 105 Statistical analysis M/cm), and expressed in µmol/100 g of tissue [18].

2.9.1 Superoxide dismutase (SOD) activity

The SOD activity was measured as follows: a certain amount of pyrogallol solution (60 mmol / 1 mmol HCl, 37 °C) was mixed with Tris-HCl buffer (pH 7.4,0.05 M, 37

°C) containing Na2EDTA(1 mmol). The total volume was adjusted by the buffer to 3000 µl, and the absorbance of the mixture without a sample (control) was measured spectrophotometrically at 325 nm every 30 s for 5 min at 37 °C. Secondly, the same amount of pyrogallol was added to the sample's homogenate, then repeated the previous steps. Enzyme activity, which corresponds to the amount of enzyme that inhibits auto-oxidation of pyrogallol by 50 % was calculated and expressed per mg of protein [18].

2.10 Histopathological examination

The morphological appearance of the kidney was examined macroscopically. The microscopic evaluation was performed by dehydrating the kidney tissue sections in ethanol, cleared in xylene, and then embedded in paraffin wax. Kidney sections were cut in 5 µm thickness and stained with hematoxylin and eosin dye. The histological features were examined by an unbiased pathologist blinded to the experimental design, performing a semi-quantitative analysis of the kidney sections slides using a light microscope. The examinations focused on renal glomerular injury and the severity of lesions was determined using scores on a scale of Grade 0: No injury, Grade 1: partial injury, and Grade 2: complete injury. This study also examined the renal tubules representing dilatation, vacuolation, and necrosis. Interstitial edema and medullary congestion were also assessed. The severity of these histological features was graded as follows: (Normal, less than 25%, 25- 50%, 50-75%, and more than 75%, and given degrees (0, 1, 2, 3, 4) respectively). At last, the presence of medullary vascular congestion was given Grade (1), and the absence was Grade (0) [19].

2.11 Statistical analysis

The statistical study was conducted by the Graph Pad Prism (9.4.1). The numerical data were expressed as (mean ± standard error of the mean SEM). Parametric data were evaluated using a one-way analysis of variance (ANOVA) test, followed by Tukey's multiple comparisons test. Categorical ordinal data was evaluated by the Mann–Whitney U test. The frequency of categorical binary data was evaluated by Fisher's exact test. The results were considered significant statically at $P<0.05$.

RESULTS

3.1 Total phenol and flavonoids contents

The total contents of phenols and flavonoids in the aqueous extract were shown in Table 1. The calibration curve of phenols and flavonoids were shown in Figure 1 and Figure 2 respectively.

Table1. Total phenol and total flavonoid contents of the cardamom aqueous extract

Total phenol (mg GAE/g)	Total flavonoids (mg QE/g)
23.44 ± 0.006	1.77 ± 0.0005
Data shown as average of triplicates ± SD, n = 3.GAE: Gallic acid equivalent, QE: Querecetin equivalent	

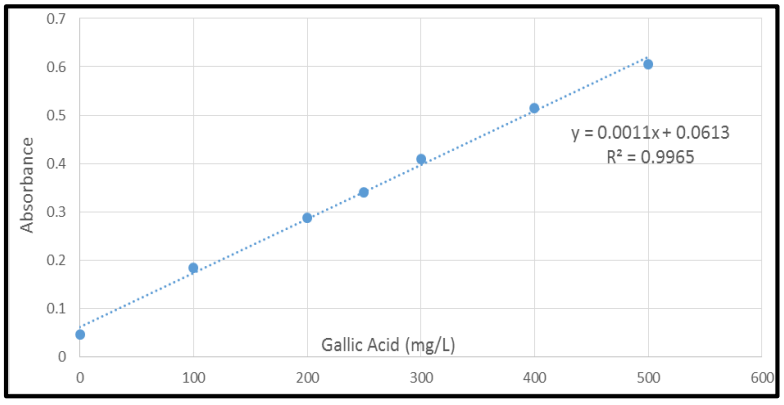

Figure 1. Calibration curve of phenol.

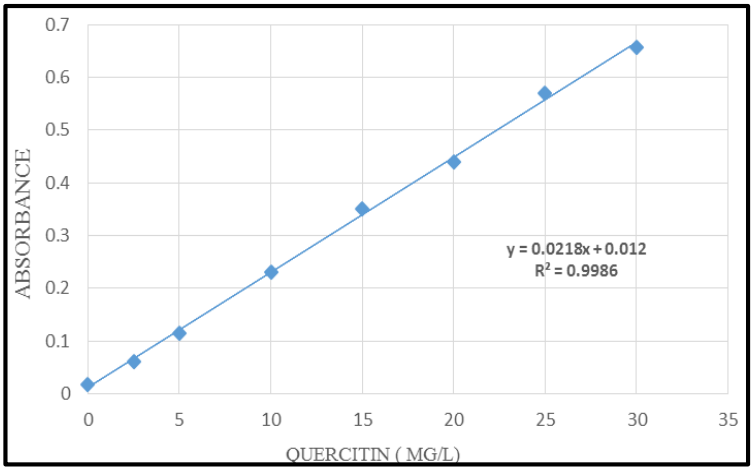

Figure 2. Calibration curve of Flavonoids

3.2 Body weight and Kidney weight/body weight ratio (%)

The body weight was reduced in the ASA group by (-14.6%) compared to the control and Card groups with statistical significances at (P<0.0001, P<0.01) respectively. While the Kidney weight/body weight ratio (%) was increased by (23.5%) compared to the control and Card groups with statistical significances at (P<0.01, P<0.05) respectively, (Table 2).

Table 2. Body weight, kidney weight and relative kidney weight of control and experimental rats.

Groups	Body weight(g)		Body weight change (%)	Absolute kidney weight (g)	Kidney weight/body weight ratio (%)
	Initial	Final			
Control	132.2±8.11	161.1±8.60	22.6±1.53	0.54±0.030	0.34±0.008
ASA	140.6±11.83	120.8±11.47	-14.6± 1.24****	0.51±0.021	0.42±0.021**
ASA + Card	129.7±9.99	118.2±8.68	-8.5±1.35##	0.41±0.030	0.35±0.023#

ASA: acetylsalicylic acid, Card: Cardamom extracts. All value represent mean ± SEM (n=8), Significance at P < 0.05. **Comparison to control group at P<0.01, **** Comparison to control group at P<0.0001.#Comparison to ASA group at P<0.05,##Comparison to ASA group at P<0.01.

3.3 Biochemical results

The levels of creatinine were significantly increased after aspirin administration compared with the control and Card group (P<0.0001.) (Figure 3). Also, the BUN was significantly increased in the ASA group (P<0.001.) (Figure 4). Cardamom extracts decreased the creatinine and BUN levels by (154.3% and 93.3%) respectively compared to the ASA group (Table 3).

Table 3. Results of creatinine, urea, lipid peroxides and SOD activity of control and experimental rats.

Groups	Serum parameters		Kidney tissue parameters	
	Creatinine (mg/dl)	Urea (mg/dl)	Lipid peroxides (µmol/ g tissue)	SOD activity
control	0.711±0.028	17.288±0.427	2.623±0.117	0.834±0.019
ASA	1.955±0.132****	39.513±2.126***	4.245±0.130****	0.563±0.038****
ASA + Card	0.787±0.033####	20.438±0.159####	2.962±0.030####	1.638±0.038####

ASA: acetylsalicylic acid, Card: Cardamom extracts. All value represent mean ± SEM (n=8), Significance at P < 0.05. ***Comparison to control group at P<0.001, **** Comparison to control group at P<0.0001. ####Comparison to ASA group at P<0.0001.

3.4 Oxidative stress biomarkers

Aspirin initiates an oxidative cascade within the kidney's tissue, which is reflected in elevating the LPO levels (38%) and declining SOD activity (49.7%) compared to the control group ($p < 0.0001$),(Table 3). Administration of cardamom aqueous extracts to the rats caused a significant reduction of the MDA levels in the kidney tissue ($p < 0.0001$) and a noticeable increase in SOD activity (65.6%) compared to the ASA group($p < 0.0001$) , (Figures 5 and 6).

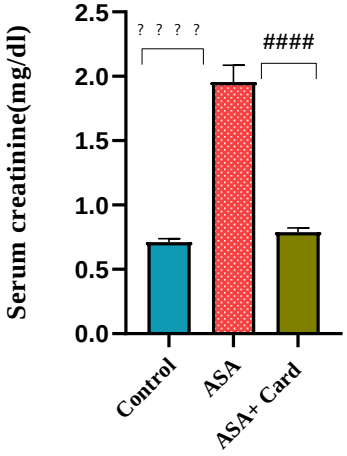

Figure 3. Serum creatinine levels in control and experimental rats groups. Values are expressed as mean±SEM

**** Comparison to normal control group at P<0.0001; ####Comparison to ASA group at P<0.0001, ASA: acetylsalicylic acid (600 mg/kg); Card: Cardamom (200 mg/kg)

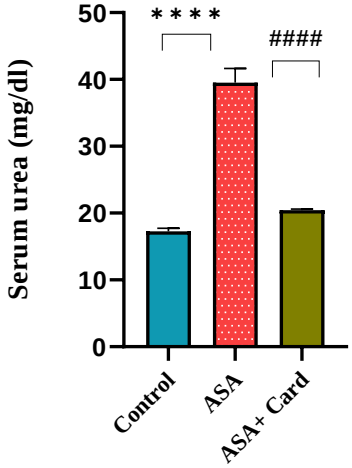

Figure 4. Serum urea levels in control and experimental rats groups. Values are expressed as mean ± SEM

**** Comparison to normal control group at P<0.0001; ####Comparison to ASA group at P<0.0001, ASA: acetylsalicylic acid (600 mg/kg); Card: Cardamom (200 mg/kg)

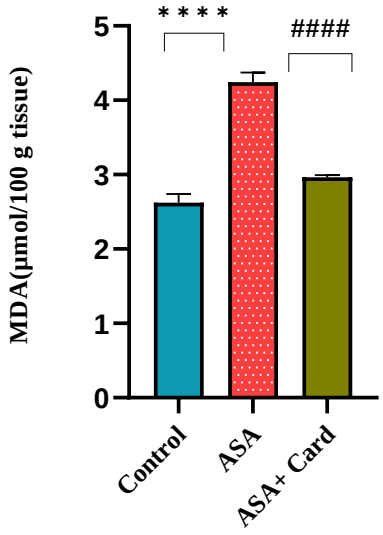

Figure 5. Lipid peroxidation levels in kidney tissue of control and experimental rats groups

Values are expressed as mean ± SEM. **** Comparison to normal control group at P<0.0001; #### Comparison to ASA group at P<0.0001, ASA: acetylsalicylic acid (600 mg/kg); Card: Cardamom (200 mg/kg).

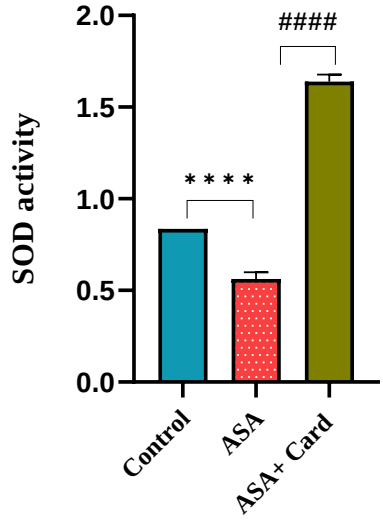

Figure 6. Superoxide dismutase (SOD) activity levels in kidney tissue of control and experimental rats groups.

Values are expressed as mean ± SEM. **** Comparison to normal control group at P<0.0001; #### Comparison to ASA group at P<0.0001, ASA: acetylsalicylic acid (600 mg/kg); Card: Cardamom (200 mg/kg)

3.5 Macroscopic examination

In the control group, the kidney had a normal macroscopic appearance with a smooth surface, while the sections showed the cortex and medulla (Figure 7:A). The kidney in the injured Group (ASA) had a bigger size and different macroscopic morphology when compared with the control group. The congestion and edema were observable, and the cortex color was pale (Figure 7: B). These macroscopic parameters and morphological changes were significantly reversed in the treatment group (Card), (Figure 7: C).

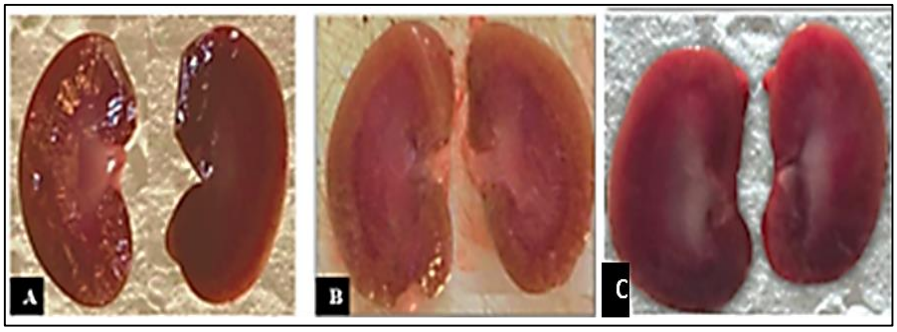

Figure 7. Macroscopic examination of the kidney sections in control and experimental rats groups. (A) Kidney with a normal macroscopic appearance in the control group. (B) Kidney is bigger and has noticeable congestion and edema with a pale cortex (ASA). (C) Morphological changes are improved in the cardamom treatment group

3.6 Microscopic Evaluation

The histopathological parameters were examined and scored (Tables: 4-5). The control group sections showed a normal glomerular appearance (Figure 6: A). Severe glomerular lesions were observed in the ASA group by the deformation of the extracellular matrix of glomerular ($p < 0.001$) compared to the control group (Figure 8: B)(Table 4). The tubule lesions showed in the ASA group, including dilatation, vacuolization, and necrosis, provided by swelling, fragmentation, and deformation of tubular epithelial cells resulting in high tubule injury scores compared to those of the control group ($p < 0.001$) (Figures 8:C, 6:D) and (Table 5). In addition, there was interstitial edema and medullary vascular congestion (Figures 8: B, 6: E), with significant static differences ($p < 0.0001$) (Table 5). While, the tubular vacuolization, necrosis, interstitial edema, and medullar congestion were significantly reduced in the cardamom group compared to the ASA group ($p < 0.01$, $p < 0.05$,$p < 0.01$, $p < 0.01$) respectively, (Figures 9:A, 7:B) and Table 4). There was a partial glomerular injury and tubule dilation with no significant statically difference in comparison with the ASA group ($p > 0.05$) and (Figure 9: C).

Table 4. Effects of aspirin and cardamom aqueous extracts on histopathological scores of a glomerular injury expressed as the frequency of injured rats in each group.

Groups	Grade 0	Grade 1	Grade 2	significance
		Histopathological Scores of glomerular injury		
Control	1 (12.5%)	7 (87.5%)	0	
ASA	0	0	8 (100%)	***
ASA +Card	2 (25%)	0	6 (75%)	ns

ASA: acetylsalicylic acid, Card: Cardamom extracts. Scores according to coverage of injury in glomerular, Grade 0: No injury. Grade 1: partial injury. Grade 2: complete injury. ns: non significance ($P > 0.05$), ***Comparison to control group at $P<0.001$.

Table 5. Effects of aspirin and cardamom aqueous extracts on histopathological scores of renal tubules (dilatation, vacuolization and necrosis), interstitial edema, and medullary congestion expressed as the frequency of injured rats in each group

Groups	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	significance
		Histopathological Scores of dilatation tubules				
Control	3 (37.5%)	3 (37.5%)	2 (25%)	0	0	
ASA	0	0	0	7 (87.5%)	1 (12.5%)	****
ASA + Card	0	0	4 (50%)	4 (50%)	0	ns
		Histopathological Scores of vacuolization tubules				
Control	3 (37.5%)	5 (62.5%)	0	0	0	
ASA	0	0	2 (25%)	5 (62.5%)	1 (12.5%)	****
ASA + Card	0	0	4 (50%)	4 (50%)	0	##
		Histopathological Scores of necrosis tubules				
Control	3 (37.5%)	5 (62.5%)	0	0	0	
ASA	0	0	0	5 (62.5%)	3 (37.5%)	****
ASA + Card	1 (12.5%)	3 (37.5%)	4 (50%)	0	0	#
		Histopathological Scores of interstitial edema				
Control	2 (25%)	6 (75%)	0	0	0	
ASA	0	0	2 (25%)	6 (75%)	0	****
ASA + Card	0	4 (50%)	4 (50%)	0	0	##
		Histopathological Scores of medullary congestion				
Control	7 (87.5%)	1 (12.5%)	0	0	0	
ASA	0	1 (12.5%)	3 (37.5%)	3 (37.5%)	1 (12.5%)	****
ASA + Card	0	0	4 (50%)	4 (50%)	0	##

ASA: acetylsalicylic acid, Card: Cardamom extracts. Scores according to percent of injury in tubular epithelium, Grade 0: normal, Grade 1(< 25%), Grade 2 (25–50%), Grade 3 (50–75%), Grade 4 (>75%). ns: non significance ($P > 0.05$), ****Comparison to control group at $P<0.0001$, ##Comparison to ASA group at $P<0.01$ #Comparison to ASA group at $P < 0.05$.

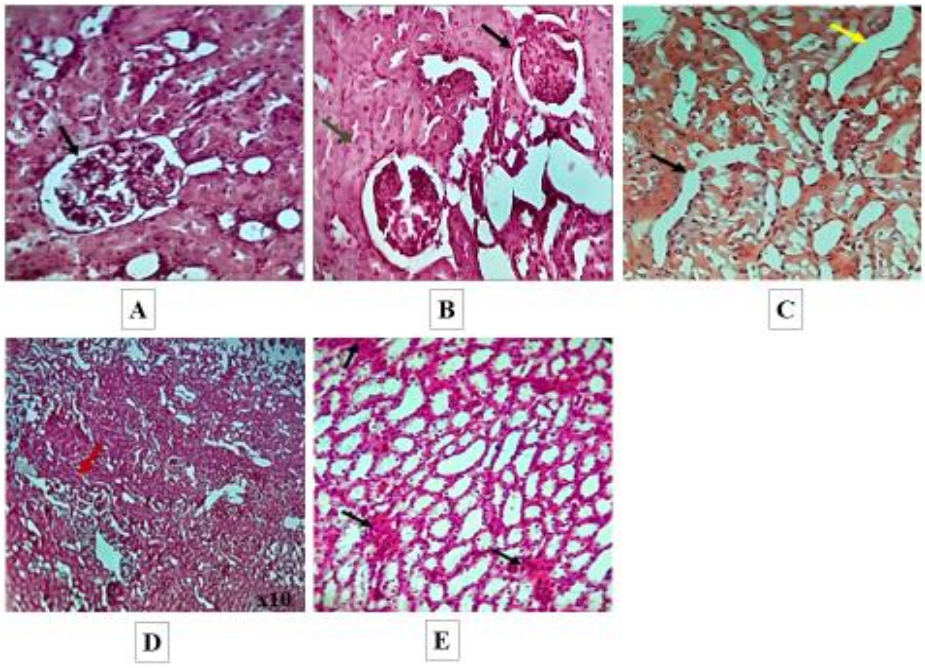

Figure 8. Histopathological changes in aspirin-induced nephropathy (600mg/kg) dose in rats (Hematoxylin and eosin x40). (A Control group): normal glomerulus, (B, C, D and E: Aspirin group). (B) Complete glomerular injury (black arrow) and interstitial edema (green arrow). (C) Abnormal dilatation (yellow arrow) and vacuolization (black arrow) of the renal tubules. (D) Total necrosis and deformation (red arrow) x10. (E) Medullary vascular congestion (black arrow).

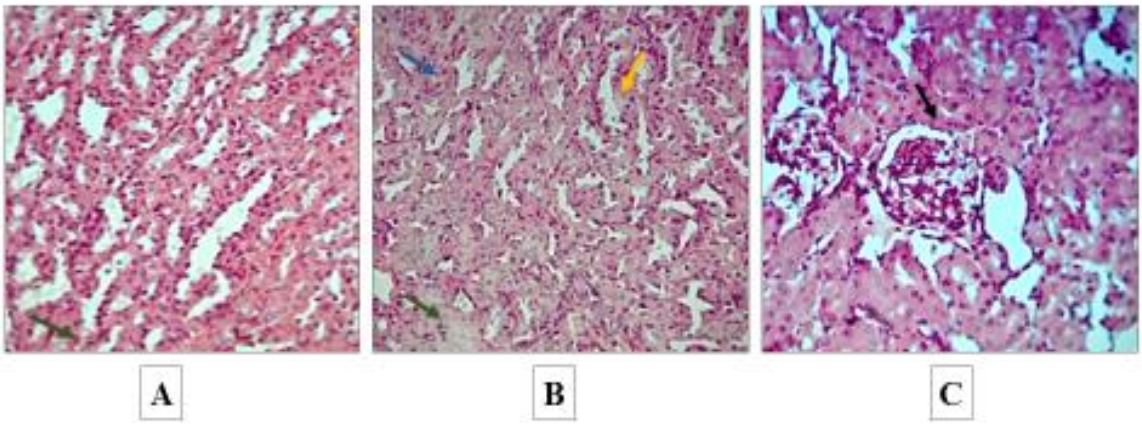

Figure 9. Effect of Cardamom aqueous extract (200 mg/Kg) on histopathological changes in (600mg/kg) dose aspirin-induced nephropathy in rats. (A, B, and C: Treatment group Card: hematoxylin and eosin x40). In (A) mild interstitial edema (green arrow) and less congestion, (B) mild tubular dilatation (yellow arrow) and vacuolization (blue arrow) in tubules, (C) partial injury to the renal glomerular

4. DISCUSSION

Due to the importance of limiting Aspirin-induced nephropathy, this study aims to investigate the effect of cardamom on controlling this damage. As the first trial was studied the aqueous cardamom extracts therapeutic efficacy on aspirin nephropathy based on biochemical and histological data.

The findings of this study revealed that aspirin kidney damage was in accord with the changes in the renal function tests and oxidative stress biomarkers. In this concern, alteration of the function of renal biochemical parameters, oxidative stress biomarkers, and histopathological studies confirmed the therapeutic effect of cardamom aqueous extract. Taking into consideration that cardamom is one of the most effective plants that scavenge free radicals and suppress their oxidative damaging effects [20]. The antioxidant activity of cardamom was attributed mainly to its high contents of phenolic and flavonoids. Thus, in our study, the total phenols and flavonoid contents in aqueous cardamom extract (Table 1). Investigation of the cardamom pharmacological effects representing in phenolic and flavonoid compounds has been suggested as possible effecting factors in previous studies [21–23].

Aspirin could induce mitochondrial dysfunction by acetylate proteins altering their morphology and leading to uncontrolled biomolecule oxidation, which increases ROS [24],and elevates plasma pro-inflammatory cytokines (IL-6, IL-8, and TNF-α), which promote oxidative stress and increases the possibility of the development of kidney diseases [25]. In addition, aspirin inhibits the mitochondrial electron transport chains and causes irreversible oxidative damage [26].The induced kidney injury and inflammation caused by aspirin administration increased kidney weight. However, because of the decreased appetite and reduction in food intake, the body-weight ratio declined compared to the control group. Therefore, the kidneys are oversensitive to reactive oxygen species (ROS) harm. Thus, increased kidney weight might be related to inflammation, edema, and oxidative stress following aspirin (600 mg/kg). These results correlated with the previous studies of [2] and [26]. In this context, cardamom elevated the body weight ratio and decreased the relative weight of the kidney in the cardamom treatment group compared to the aspirin group, which attributed mainly to flavonoids mechanism in

suppressing the NF-κB inflammation and preventing the expression of proinflammatory cytokines (IL-6, IL-1β, TNFα) [27]. That partly disagrees with the study of Aboelnaga with cardamom reduced body weight, while it is compatible with our results in decreasing relative kidney weight [28].

Creatinine and urea are still the most usable and significant indicators in renal function estimation [29]. Creatinine and urea levels were significantly elevated in the aspirin group compared to the control group, in agreement with the study of Ogunjemite et al. In a way, aspirin inhibits the synthesis of PGE2 and PGI2, causing a decrease in renal blood flow that may lead to impaired renal function, reflected by increased creatinine and urea levels [30]. The cardamom reversed the harmful effect of aspirin by improving kidney functions represented in creatinine and urea, which were reported earlier in other models of renal impairment induced by gentamicin by [13] and in a previous study by [20] in doxorubicin nephrotoxicity.

Renal ischemia caused by aspirin may stimulate the production of ROS [24], which initiates lipid peroxidation observed by elevating MDA levels and altering the endogenous antioxidant SOD functions and concentrations too [31]. Accordingly, to these cellular variations that confirmed renal tissue injury, aspirin caused a significant increase in MDA levels and a decrease in SOD activity in the current study, compatible with the results reported by [32]. On the other hand, there was a significant increase in SOD levels and a decrease in MDA levels in the cardamom treatment group. Due to its richness in potent phenolic and flavonoid antioxidant compounds suppressing lipid peroxidation and neutralizing the free radicals activity, in agreement with a previous study by [33] that detected the protective role of cardamom against diethylnitrosamine-induced oxidative stress in the kidney. The histopathological evaluation supported biochemical findings and indicated that cardamom has an antioxidant effect on the renal damage induced by aspirin. Whereas in our study, aspirin-induced renal damage was associated with tubular injuries (dilatation, vacuolization, and necrosis) and glomerular degeneration. These results are consistent with the results of previous studies [2,34].

The current study indicated that cardamom moderates tubular injuries and reduces congestion compared to the aspirin group. According to Khattab et al previous study, cardamom caused a histological improvement in renal tubule vacuolization, which could be attributed to its antioxidant and free radical scavenging properties [35].

CONCLUSIONS

In summary, the current study demonstrated the therapeutic effect of cardamom against aspirin-induced nephropathy. Cardamom has reduced kidney injury by improving the histological, biochemical, and kidney oxidative stress parameters. The antioxidant bioactivity of cardamom is explained by its high content of phenols and flavonoids.

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تقييم فعاليّة خُلاصة حب الهال في علاجِ الاعتِلالِ الكُلويّ المُحدَث بالأَسبرين عِندَ جرذانِ التجربة

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ملخص

يعدّ الاعتلال الكلوي بمسكنات الألم أحد الاختلاطات الناتجة عن الاستخدام المفرط والمزمن للمسكنات، وخاصةً التي تصرف بدون وصفة طبية مثل الأسبرين. تهدف هذه الدارسة إلى تحرّي التأثير العلاجيّ لخلاصة الهال على الاعتلال الكلويّ المُحدث بالأسبرين عند جرذان التجربة. وُزعَ 24 جرذاً إناثاً من فصيلة Albino Wistar على ثلاث مجموعات (8 حيوانات/مجموعة): (Control) المجموعة الشاهدة الطبيعية، (ASA) الأسبرين (600 ملغ/كغ/اليوم مدّة 4 أيام)، (ASA + Card 100): الأسبرين + خلاصة الهال (100 ملغ/كغ/اليوم مدّة 7 أيام)، و (ASA+ Card 200): الأسبرين + خلاصة الهال (200 ملغ/كغ/اليوم مدّة 7 أيام. حُضِّرَت الخلاصة المائية للهال. حُسب محتواها من الفينولات والفلافونوئيدات. حُسبت نسبة وزن الكلية/ وزن الجسم، وقيّست مستويات البولة والكرياتينين المصليين. قُيّمتْ مستويات فوق أكاسيد الشحوم بقياس تراكيز Malondialdehyde (MDA) وفعالية أنزيم Superoxide dismutase (SOD) في نسيج الكلية، بالإضافة للفحوص النسيجية. قُيّمت البيانات المعلمية باستخدام اختبار التباين الأحادي (ANOVA) One-way analysis of variance متبوعاً باختبار Tukey's. والبيانات اللامعلمية قُيّمت باختبار Mann–Whitney متبوعاً باختبار Ficher، عُدَّت النتائج جوهرية عندP<0.05. قُدِّرَ محتوى الفينولات 23.4 مغ مكافئ حمض الغاليك/غ خلاصة جافة، والفلافونوئيدات 1.77 مغ مكافئ كيرستين/غ خلاصة جافة. لوحظ ارتفاعاً معتداً به إحصائياً في نسبة وزن الكلية/ وزن الجسم، وفي البولة والكرياتينين المصليين، وتراكيز MDA، في حين انخفضت فعالية SOD وذلك بمقارنة مجموعة الأسبرين مع المجموعة الشاهدة الطبيعية. كما أظهر الفحص النسيجي أذيّاتٍ نبيبية وكبيبية معتدّاً بها إحصائياً. لوحظ تحسّناً جوهرياً في معظم المعالم المصليّة والنسيجيّة لدى مجوعة العلاج Card 200 لدى مقارنتها مع مجموعة الأسبرين. أظهرت خلاصة نبات الهال (200 مغ/كغ) فعالية علاجيّة تِجاه الاعتلال الكلويّ المُحدث من خلال تحسن وظائف الكلية، والمعالم الأنزيمية والنسيجية، وذلك بفضل فعاليته المضادة لحالة الإجهاد التأكسدي المُحدث بالأسبرين.

الكلمات الدالة: الكلية، الاعتلال الكلوي، مضادات الالتهاب اللاستيروئيدية، الأسبرين، خلاصة الهال، الإجهاد التأكسدي.

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