Hydroethanolic Leaf Extract of *Murraya Koenigii*: Phytochemical Constituents and Biological Evaluation of its Toxicity and Antipyretic Activity in Wistar Albino Rats

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ABSTRACT

Background: Fever, characterized by an elevated body temperature beyond the normal range, necessitates effective management. Traditional therapies rooted in indigenous knowledge prove effective, in addressing fever-related conditions for optimal well-being. This study explores the antipyretic potential of *Murraya koenigii*, a plant deeply rooted in traditional practices in Nepal.

Materials and Methods: The hydroethanol leaf extract of *Murraya koenigii* was subjected to phytochemical screening and acute toxicity assessment, followed by *I*n vivo antipyretic effects evaluated in male Wistar Albino rats using a yeast-induced fever model.

Results: Phytochemical analysis revealed the presence of bioactive compounds such as saponins, flavonoids, glycosides, phenols, tannins, and alkaloids. The acute toxicity study demonstrated the safety of *Murraya koenigii* extract up to 5000 mg/kg, highlighting its wide safety margin. *In vivo* antipyretics evaluation showed a significant ($p< 0.05$) temperature reduction at time 90 and 120 minutes by *Murraya koenigii* hydroethanolic extract (250mg/kg), comparable to the negative control group.

Conclusion: In conclusion, this study provides valuable insights into the phytochemical profile, safety, and antipyretics properties of *Murraya koenigii*, supporting its traditional use for fever management.

Keywords: *Murraya koenigii*, antipyretics, phytochemical, acute toxicity.

1. INTRODUCTION

Fever, a frequent increase in body temperature, is characterized by an elevated body temperature beyond the normal range (36.5-37.5°C) due to a raised hypothalamic set point [1]. The production of prostaglandins E2 in the preoptic area of the hypothalamus is necessary for altering neuronal firing rates, ultimately causing fever[2]. Antipyretic medication works by inhibiting cyclooxygenase-2 (COX-2) expression, which decreases the production of prostaglandin E2 (PGE2), the chief mediator of fever. Non-steroidal anti-inflammatory drugs (NSAIDs) like Diclofenac, aspirin and antipyretic drug are commonly used as antipyretic medication[3]. Due to the widespread adverse effects of synthetic medications, there has been a notable increase in interest in natural goods, leading to rise in studies aimed at examining their possible advantages[4,5]. The use of natural plant products as therapeutic solutions for fever and inflammation has become more well-known over the last 20 years due to continued study and the relatively low incidence of side effects when compared to manufactured drugs [6,7].

In Nepal, traditional methods for fever often involve the utilization of varieties medicinal plant species. One

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such plant is *Murraya koenigii (M. koenigii)*, belonging to the Rutaceae family. Widely distributed in tropical and subtropical regions in Nepal. It has been traditionally employed in diverse cultural practices for its medicinal properties including hair care, fever control, digestive aid, diabetics management, stimulant, stomachic, analgesic, and therapeutic qualities that may be used to treat bug bites, diarrhea, and dysentery in addition to its antidepressant and anti-inflammatory qualities[8 9]. The fragrant leaves of Murraya koenigii are well-known for their usage as a culinary spice. Its powerful, characteristic perfume is mostly caused by four chemical compounds: O-phellandrene, p-caryophyllene, p-elemene, and p-gurjunene. Additionally, this plant has large concentrations of carbazole alkaloids, including isomurrayazoline, mahanine, koenine, koenigine, koenidine, and koenimbine[10].

Despite its extensive use, scientific investigations into the phytochemical constituents, acute toxicity profile, and antipyretic potency of *Murraya* species are very limited. Therefore, the study was designed to examine the antipyretic effects of *M. koenigii* leaf hydroethanolic extracts in rat models.The findings of this study aim to bridge the gap between traditional knowledge and scientific validation, providing valuable insights into the pharmacological properties of *M. koenigii* and its potential role in managing fever-related conditions.

2. MATERIALS AND METHODS

2.1 Plant sample collection, identification, and preparation

The leaves of *M. koenigii* were collected from the local place i.e., Bharatpur-2, Chitwan, Nepal in May 2016. The samples were identified and authenticated by the Agribotany Department of Agriculture and Forestry University of Rampur, Chitwan, Nepal, and were assigned with voucher specimen' number AG0223. The collected leaves were shade dried for 15 days and ground to coarse particles using a mechanical grinder.

2.2 Extraction

The powered leaves of *M. koenigii* were cold macerated for 24 hours in 70% ethanol in the ratio of 1:8 i.e., 8ml of ethanol was used for each g of *M. koenigii* powder. Then supernatant was filtered and separated from marc. The obtain *M. koenigii* extract was concentrated under rotatory evaporator. The concentrated slurry was then kept for five days in a vacuum desiccator to ensure complete dryness[11].

2.3 Phytochemical screening

The hydroethanolic extracts of *M. koenigii* leaves were assessed for alkaloids, saponins, tannins, flavonoids, glycosides, and phenolic compounds, using Mayers, Froath formation, Ferric chloride, Shinoda, Keller-Killiani and Lead acetate test method respectively [12 13 14].

2.4 Experimental animals

Male Wistar Albino rats (2-3 months old, average weight 150 g) were bought from the animal house department of plant resources, Kathmandu, Nepal, and were kept in polyethylene cages at 25 ± 2°C, 40–60% humidity, with a 12-hour light-dark cycle, and provided standard diet and water ad libitum housed at primate facility of the Chitwan medical college, Nepal. Ethical approval was obtained from Institution Research Committee (IRC) of Chitwan Medical College for the experiment with reference no. CMCIRC465.

2.5 Experimental design

2.5.1 Acute toxicity study

Male Wistar Albino rats were used to assess the toxicity of *Murraya koenigii* leaves hydroethanolic extract (MKLHE) following the guidelines outlined by OECD (Organization, for Economic Co-operation and Development) guideline 423. The rats were divided into four groups, each consisting of six rats and underwent a 12-hour fasting period while having access to water. The control group was given distilled water at dose 10 ml/kg whereas the other three groups received *M. koenigii* extract was dissolved in distilled water and administered at doses of 1000 mg/kg, 3000 mg/kg and 5000 mg/kg respectively.

Throughout 30 minutes, continuous monitoring was conducted to observe any changes in behavior, food and water intake as mortality. Subsequently, intermittent checks were performed from 4 to 24 hours after administration. These observations continued for 14 days[15].

2.5.2 Antipyretic study

Twenty-seven male wistar rats (140-150 g) were randomly divided into 3 groups and fasted overnight before the experiment with free access to water. The normal body temperature of each rat was measured rectally using a lubricated thermometer. After measuring the basal rectal temperature. A 15% baker's yeast suspension in 0.9% saline was prepared and injected subcutaneously to the each of rats. Eighteen hours after bakers's yeast injection, the animals were again restrained for rectal temperature recording, as described previously. Only rats that showed an increase in temperature of at least 1°C were used for this study. The MKHEE at the doses of 250 mg/kg were administered orally to one groups of animals. The negative control group received an 1ml of vehicle (0.9% saline), and the positive control group received paracetamol 150mg/kg) orally. Rectal temperature was measured at 30 minutes intervals for 2 hours after the extract/drug administration[16].

2.6 Data management and statistical analysis

The data are shown as mean ± standard deviation (n = 9). Data management and inferential analysis was done by SPSS version 16 software. *P values* ≤ *0.05* were regarded as a significant indicator.

3. RESULTS

3.1 Qualitative phytochemical analysis

Table 1, shows the phytochemical test results from *M. koenigii*. This table displays the presence of active phytochemical constituents, which are organic substances found in an extract.

Table 1. Phytochemical constituents present in M. koenigii

S.no	Test	Results
1.	Saponins	+
2.	Flavonoid	+
3.	Glycosides	+
4.	Phenols	+
5.	Tannins	+
6.	Alkaloids	+

+ = Presence**, -** = Absence

3.2Acute toxicity study

The acute toxicity study of *M. koenigii* extract in male Wistar Albino rats revealed no mortality or adverse reactions at doses up to 5000 mg/kg. Continuous monitoring for 30 minutes and subsequent observations over 14 days showed no signs of diarrhea, mortality, hair fall, or behavioral changes, indicating the safety of *M. koenigii* within the tested dose range.

3.3 In-vivo Antipyretic Effects

As shown in Table 2 over 120 minutes, MKLHE demonstrated a potential antipyretic effect in albino rats. At 90 and 120 minutes, MKLHE significantly ($p < 0.05$) reduced body temperature compared to the negative control (distilled water) and exhibited comparable efficacy to the standard (paracetamol), suggesting its promising role in fever management.

Table 2. In vivo antipyretic effect at different time intervals

Time (Minute)	Water for injection (0.9% saline) 1ml	Standard (Paracetamol) 150mg/kg	MKLHE 250mg/kg
0	101±0.4	100.83±0.50	100.94±0.45
30	101.23±0.39	100.1±0.45	101.3±0.3
60	101.2±0.33	99.16±0.25	99.33±0.25
90	100.73±0.2	98.33±0.15*	98.63±0.16*
120	100.4±0.13	97.96±0.11*	98.2±0.11*

Note: * indicates *P value ≤ 0.05* level of significance when compared with negative control

4. DISCUSSION

The qualitative phytochemical analysis of the hydro ethanol leaf extract of *M. koenigii* revealed the presence of various active constituents, including saponins, flavonoids, glycosides, phenols, tannins, and alkaloids (Table 1). A similar finding was reported by Yohanes et al, in their analysis of *Murray* species. These compounds are known for their diverse pharmacological activities, and their presence aligns with traditional knowledge and supports the exploration of *M. koenigii* for medicinal purposes[17]. Previous studies by Balakrishnan et. al have reported mahanine, mahanimbine, isomahanine, koenimbine, girinimbine, and isolongifolene as the principal chemical constituents present in Murraya species, showcasing pharmacological activity [18].

The acute toxicity study demonstrated the safety of *M. koenigii* extract in male Wistar Albino rats up to a dose of 5000 mg/kg. The absence of mortality or adverse reactions over a 14-day observation period suggests a wide safety margin. This finding is consistent with the finding of Menezes et al [19], who also observed no mortality or adverse reactions up to 5000mg/kg in their study on the safety profile of *M. panculata* .The wide safety margin observed in the present study reinforces the potential therapeutic use of the *M. koenigii* leaves.

The *in vivo* antipyretic effect of *Murray koenigii* leaves hydroethanolic extract was evaluated in Wistrar albino rats, indicating a reduction in body temperature at 90 and 120 minutes, comparable to the standard drug, paracetamol (150mg/kg) (Table 2). This finding supports the traditional use of *M. koenigii* for fever management and suggests its potential as a natural antipyretic agent. This data closely aligns with the work of Forkuo et al [20], who has reported a similar antipyretic effect with *M. koenigii* exotic a methanol leaf extract in their study. The comparison with 150 mg/kg paracetamol strengthens the credibility of the present finding, suggesting the potential of *M. koenigii* as a natural antipyretic agent. The presence of alkaloids, terpenoids and flavonoids in *M. koenigii*, as revealed in the phytochemical analysis, aligns with the observed antipyretic effect. Alkaloids, known for their ability to suppress cyclooxygenase (COX) enzymes, may contribute to the reduction of body temperature by modulation of inflammatory pathways, inhibiting of cyclooxygenase enzymes in the brain ,and effect on the central nervous system to regulate body temperature in a mechanism similar to the mechanism of Saction of paracetamol[21, 22]. The potential role of alkaloids in modulating fever might reinforce the traditional use of *M. koenigii* leaves for fever-related conditions[23]. As previous study carried out by Forkuo et al, on *Murraya exotica* (L.) found that at dose 300mg/kg the reduction in rectal temperature was 66.42% showcasing the antipyretic effect of *Murraya exotica* (L.) leaves extract[24].

5. CONCLUSION

Hence the comprehensive study highlights the phytochemical profile, safety, and antipyretic properties of *M. koenigii*. The results support its traditional use in fever management and suggest its potential as a valuable

therapeutic agent. This study also support the traditional use *M. koenigii* for fever management. Further investigation into the molecular mechanism of alkaloids obtained from *M. koenigii* and clinical applications of *M. koenigii* is necessary to justify its medicinal potential.

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Conflict of Interest

The authors declare that they have no known competing financial interest or personal relationship that could have appeared to influence the work reported in this paper.

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مستخلص الأوراق الهيدروإيثنولية من *Murraya koenigii*: المكونات الكيميائية النباتية والتقييم البيولوجي لسميتها ونشاطها الخافض للحرارة في فئران ويستار ألبينو

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ملخص

الخلفية: الحمى، التي تتميز بارتفاع درجة حرارة الجسم إلى ما هو أبعد من المعدل الطبيعي، تتطلب إدارة فعالة .تثبت العلاجات التقليدية المتجذرة في معارف السكان الأصليين فعاليتها، حيث تعالج الظروف المرتبطة بالحمى لتحقيق الرفاهية المثلى. تستكشف هذه الدراسة إمكانات خافض للحرارة لنبات Murraya koenigii، وهو نبات متجذر بعمق في الممارسات التقليدية في نيبال .

المواد والطرق: تم إخضاع مستخلص أوراق الهيدروإيثانول من Murraya koenigii للفحص الكيميائي النباتي وتقييم السمية الحادة متبوعًا بتأثيرات خافضة للحرارة في الجسم الحي تم تقييمها في ذكور فئران ويستار ألبينو باستخدام نموذج الحمى الناجم عن الخميرة.

النتائج: كشف التحليل الكيميائي النباتي عن وجود مركبات نشطة بيولوجيا مثل الصابونين والفلافونويدات والجليكوسيدات والفينولات والعفص والقلويدات .أظهرت دراسة السمية الحادة سلامة مستخلص موراي كونيجي حتى 5000ملغم/كغم، مما يسلط الضوء على هامش الأمان الواسع .أظهر تقييم خافضات الحرارة في الجسم الحي انخفاضًا كبيرًا في درجة الحرارة ($p< 0.05$) في الوقت 90و 120دقيقة بواسطة مستخلص Murraya koenigii الهيدروإيثانوليك (250)مجم/كجم، مقارنة بمجموعة التحكم السلبية.

الخلاصة: في الختام، توفر هذه الدراسة رؤى قيمة حول خصائص المواد الكيميائية النباتية والسلامة وخافضات الحرارة في Murraya koenigii، مما يدعم استخدامها التقليدي لإدارة الحمى.

الكلمات الدالة: موراي كونيجي، خافضات الحرارة، كيميائية نباتية، سمية حادة.

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