

Analgesic and Anti-Inflammatory Effects of Ethanolic Seed Extract of *Hunteria umbellata* in Murine Models

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Abstract

Hunteria umbellata seeds are traditionally used to manage pain and inflammatory disorders. This study evaluated the acute oral toxicity, analgesic activity, and anti-inflammatory activity of an ethanolic extract of *H. umbellata* seeds in murine models. Acute oral toxicity was assessed using Lorke's method. Analgesic activity was evaluated using acetic acid-induced writhing, formalin-induced nociception, and hot plate assays, while anti-inflammatory activity was assessed using carrageenan- and egg albumin-induced paw oedema models. No observable toxicity was reported at doses up to 5,000 mg/kg. The extract demonstrated significant analgesic and anti-inflammatory activities, reducing acetic acid-induced writhing and inhibiting both the neurogenic and inflammatory phases of formalin-induced nociception. It also prolonged reaction latency in the hot plate assay, suggesting a central component to its analgesic effects. Anti-inflammatory activity was demonstrated by reduced paw oedema in both the carrageenan and egg albumin models. These findings demonstrate the analgesic and anti-inflammatory potential of the ethanolic seed extract and provide preclinical support for the traditional use of *H. umbellata* seeds in managing pain and inflammatory disorders.

Keywords: *Hunteria umbellata*; Analgesic Activity; Anti-Inflammatory Activity; Acute Oral Toxicity; Nociception

INTRODUCTION

Pain and inflammation are interconnected biological processes that play vital roles in the body's defense mechanisms and tissue repair. Acute inflammation triggers protective responses against infections and tissue injuries, whereas chronic or unregulated inflammatory reactions can lead to persistent conditions such as arthritis, cardiovascular diseases, diabetes, cancer, and autoimmune disorders (Medzhitov, 2021). Pain, whether acute or chronic, remains one of the leading causes of disability and medical consultation globally, imposing a substantial socioeconomic burden (Harris, 2024).

Conventional pharmacological agents, including non-steroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and opioids, are widely used for the management of pain and inflammatory conditions. However, despite their therapeutic efficacy, their prolonged use is often associated with adverse effects, including gastrointestinal ulceration, renal toxicity, cardiovascular complications, immunosuppression, and the potential for dependence (Risser *et al.*, 2009; Biancuzzi *et al.*, 2022). Consequently, these limitations associated with conventional therapies have intensified the research into medicinal plants as potential sources of safer and more effective therapeutic agents.

Natural products contain diverse phytochemicals such as flavonoids, alkaloids, tannins, phenolics, and saponins, which have demonstrated analgesic and anti-inflammatory activities through antioxidant effects and modulation of inflammatory mediators (Jahromi *et al.*, 2021). Studies by Ahsan *et al.*, (2023) on *Citrus maxima* seed extract demonstrated potent analgesic and anti-inflammatory effects in Swiss albino mice, highlighting the role of bioactive compounds in modulating pain perception and inflammatory responses. These findings support the growing interest in phytotherapeutics as alternatives for managing inflammation and pain.

Hunteria umbellata is a small glabrous tree belonging to the family *Apocynaceae* and is widely distributed across the tropical forests of West and Central Africa, including Nigeria, Cameroon, Ghana, Senegal, Gabon, and the Congo region (Nyawuame & Gills, 1991). Uka *et al.* (2025) reported that the seeds are locally known as "Asewa" among the Ibibio and Efik ethnic groups of Nigeria and contain several bioactive constituents, including alkaloids, flavonoids, tannins, phenolic compounds, terpenoids, and saponins, which may contribute to their pharmacological activities.

Various parts of the plant, particularly the seeds, leaves, bark, and roots, are widely used in African ethnomedicine for the treatment of fever, pain, inflammation, gastrointestinal disorders, infections, and reproductive ailments (Ezuruike & Prieto, 2014). Although *Hunteria umbellata* has been traditionally employed for the treatment of painful and inflammatory conditions, scientific evidence supporting its analgesic and anti-inflammatory activities remains limited. Therefore, the present study was designed to evaluate the acute toxicity profile, analgesic activity, and anti-inflammatory effects of the ethanolic seed extract of *Hunteria umbellata* using established murine models.

MATERIALS AND METHODS

Collection and Extraction of Plant Material

Hunteria umbellata mature fruits were obtained from the Pharmacy Plantation at the University of Uyo, Uyo, Akwa Ibom State, Nigeria. The plant was authenticated by Mrs. Emmanuella Udoma, a taxonomist in the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo. A voucher specimen (Voucher No.: UUPH I) was deposited at the Departmental herbarium. Manually dehusked seeds were washed with clean water, shade-dried, and pulverized into a coarse powder. The powdered material was sequentially extracted using ethanol. The filtrate obtained was concentrated to dryness in a water bath maintained at 40 °C to remove the solvent completely and obtain the crude extract as previously described (Uka *et al.*, 2025).

Drugs, chemicals, and instruments

Carrageenan, acetylsalicylic acid (ASA), and dexamethasone were obtained from Sigma Aldrich (USA); acetic acid from Merck (Darmstadt, Germany); formalin from Nihon Shiyaku Industries (Japan); ethanol from JHD (China); and fresh egg albumin from Merck (Germany). Equipment included a VTCL Solitaire mixer grinder (VTCL, India), a digital Vernier caliper (V. Tech, India), and an electronic weighing scale (Ohaus Corporation, USA; model Scout Pro SPU-202), all housed in the Pharmacognosy Laboratory, University of Uyo, Nigeria. Unless otherwise specified, all other chemicals and reagents were of analytical grade and sourced locally.

Experimental Animals

A total of one hundred and twenty-five Swiss albino mice (17-29 g) were sourced from the Department of Biochemistry animal facility, University of Calabar, Nigeria. The animals were allowed a two-week acclimatization period under controlled laboratory conditions, with unrestricted access to water and standard chow. All experimental procedures were approved by the Animal Ethics Committee, Faculty of Basic Medical Sciences, University of Calabar (Approval No. 39BCM3125) and conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 2011).

Acute Toxicity Studies

Acute oral toxicity was assessed following Lorke's (1983) protocol, with slight modifications. The study was conducted in two phases. In the first phase, three groups of mice ($n = 3$) per group received doses of 10, 100, and 1000 mg/kg of the extract. Animals were monitored for 24 hours for behavioural or physical signs of toxicity. In the second phase, fresh groups of mice were administered higher doses (1600, 2900, and 5000 mg/kg) and observed similarly. The median lethal dose (LD_{50}) was calculated using the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

Where:

D_0 = highest dose without mortality.

D_{100} = lowest dose that caused complete mortality.

Determination of the Analgesic Activity of Seed Extract of *Hunteria umbellata*

The analgesic effect of the seed extract of *Hunteria umbellata* was evaluated using acetic acid-induced abdominal writhing, formalin-induced paw licking, and the hot plate test models.

Acetic acid-induced Abdominal Writhing

The acetic acid-induced writhing model was used to assess the peripheral analgesic activity of the seed extract of *Hunteria umbellata* against abdominal writhing, following the modified procedures described by Uka *et al.* (2020).

Twenty-five mice (17–25 g) were randomly divided into five groups ($n = 5$). Before the test, the mice were fasted for 24 hours but allowed free access to water. Groups 1–3 received intraperitoneal doses of 500, 1000, and 1500 mg/kg of the extract; group 4 received distilled water (10 mL/kg), and group 5 was treated with aspirin (100 mg/kg). One hour later, 0.2 mL of 3% acetic acid was injected intraperitoneally to induce writhing. The number of abdominal contractions was recorded at 5-minute intervals for 30 minutes as described by Alibabaei *et al.* (2010).

Percentage Inhibition of Acetic acid-induced Abdominal Writhing

Analgesic activity was expressed as percentage inhibition in writhing compared to the control group, using the formula:

$$\text{Percentage inhibition (\%)} = \frac{W_c - W_t}{W_c} \times 100$$

Where:

W_c = Mean writhes in the control group

W_t = Mean writhes in the test groups.

Formalin-Induced Paw Licking in Mice

The formalin-induced paw licking model was employed to evaluate both neurogenic and inflammatory pain responses following administration of the seed extract of *Hunteria umbellata* based on the method described by Uka *et al.* (2020), with minor modifications.

Twenty-five mice of both sexes (17–27 g) were divided into five groups ($n = 5$) and treated intraperitoneally with extract doses (500, 1000, 1500 mg/kg), distilled water, or aspirin. Food was withheld for 24 hours prior to the experiment, while water was provided ad libitum. Thirty minutes after treatment, 0.02 mL of 2.5% formalin solution was injected into the right hind paw. Paw licking duration was recorded during the early phase (0–5 min), associated with direct nociceptor stimulation, and the late phase (15–30 min), linked to inflammatory mediators such as histamine, serotonin, bradykinin, and prostaglandins (Dubuisson & Dennis, 1977; Hunskaar & Hole, 1987).

Percentage Inhibition of Formalin-induced Paw Licking in Mice

Percentage inhibition was calculated by comparing the treated groups with the control using the

$$\text{Percentage inhibition (\%)} = \frac{D_o - D_t}{D_o} \times 100$$

Where:

D_o : mean paw licks in control

D_t : mean paw licks in treated groups.

Hot Plate Test in Mice

Central analgesic activity was evaluated using the hot plate assay described by Eddy & Leimbach (1953) with slight modifications. Mice (17–26 g) were divided into five groups ($n = 5$) and treated intraperitoneally with extract doses (500, 1000, 1500 mg/kg), distilled water, and dexamethasone (100 mg/kg). Thirty minutes after treatment, each mouse was placed on a hot plate maintained at $45 \pm 1^\circ\text{C}$, and the latency to nociceptive responses (paw licking, withdrawal, or jumping) was recorded. A cutoff time of 30 seconds was applied to prevent tissue injury, following the procedure outlined by Kulkarni (2014). Increased reaction latency relative to controls was taken as evidence of central analgesic activity.

Percentage Inhibition of Hot Plate Test in Mice

The percentage change in response time used as a measure of analgesic effectiveness was calculated using the formula outlined by Eddy and Leimbach (1953). The percentage inhibition of nociception was calculated using the formula:

$$\text{Percentage inhibition (\%)} = \frac{T_t - T_c}{T_c} \times 100$$

Where:

T_t = Reaction time after administering the analgesic

T_c = Reaction time without drug treatment

Determination of Anti-Inflammatory Activity of *Hunteria umbellata* Seed Extract

The anti-inflammatory potential of the seed extract was evaluated using two established models: carrageenan-induced paw oedema and egg albumin-induced paw oedema. These assays were selected to assess both acute and sub-acute inflammatory responses.

Carrageenan-induced Paw Oedema

Inflammation was assessed by monitoring paw swelling, which served as the primary indicator of the inflammatory response (Yada *et al.*, 2025). To evaluate the acute inflammatory reaction and the anti-inflammatory potential of the test extract, the carrageenan-induced paw oedema model was applied, following the protocol of Winter *et al.* (1962) with slight modifications.

This model progresses through three distinct phases: an early stage mediated by histamine and serotonin release (0–1.5 h), a second stage driven by kinins (1.5–2.5 h), and a later stage involving prostaglandins, thromboxane A₂, and resolvins (after 3 h) (Mohan, 2010).

A total of 25 mature mice (17 and 28 g) were randomly assigned to five groups (n = 5). Groups 1–3 received extract doses of 500, 1000, and 1500 mg/kg respectively. Group 4 received distilled water (10 mL/kg) as the negative control, and group 5 received aspirin (100 mg/kg) as the reference drug. One hour after treatment, 0.1 mL of a 1% carrageenan suspension was injected into the right hind paw. Paw thickness was recorded at baseline and subsequently at 0.5, 1, 2, 3, 4, and 5 hours post-injection using a digital vernier caliper, following the procedure outlined by Hess & Milonig (1972). Oedema was expressed as the difference in thickness between the treated and untreated paws.

$$\text{Mean paw oedema (mm)} = T_t - T_u$$

Where:

T_u = thickness of the untreated paw

T_t = thickness of the treated (inflamed) paw

Percentage Inhibition of Carrageenan-Induced Paw Oedema

The percentage inhibition of carrageenan-induced paw oedema was calculated as the reduction in paw swelling compared to the control group, using the formula described by Balamurugan *et al.* (2012)

$$\text{Percentage Inhibition (\%)} = \frac{V_c - V_t}{V_c} \times 100$$

Where:

V_c = mean paw oedema in the control group

V_t = mean paw oedema in the treated group

Egg Albumin-Induced Paw Oedema

The egg albumin-induced paw oedema model is an established method for assessing acute inflammation, comparable to the carrageenan model but using raw, undiluted egg albumin as the irritant (Eze *et al.*, 2023). The procedure followed Hess and Milonig (1972) with slight modifications.

Twenty-five Swiss albino mice (19–29 g) were randomly divided into five groups ($n = 5$). Groups 1–3 received intraperitoneal doses of 500, 1000, and 1500 mg/kg of seed extract; group 4 was given distilled water (10 mL/kg); and group 5 received aspirin (100 mg/kg). One hour after treatment, acute inflammation was induced by injecting 0.1 mL of freshly prepared egg albumin into the subplantar surface of the right hind paw. Paw thickness was measured at baseline and at 0.5, 1, 2, 3, 4, and 5 hours post-injection using a digital vernier caliper. Oedema was calculated as the difference between the treated (right) and untreated (left) hind paw at each time point.

$$\text{Mean paw oedema (mm)} = T_t - T_u$$

Where:

T_u = thickness of the untreated paw

T_t = thickness of the treated (inflamed) paw

Percentage Inhibition of Egg Albumin-Induced Oedema

The anti-inflammatory effect was expressed as a percentage inhibition relative to the control group, using the formula (Winter *et al.*, 1962):

$$\text{Percentage Inhibition (\%)} = \frac{E_c - E_t}{E_c} \times 100$$

Where:

E_c = mean paw oedema in the control group

E_t = mean paw oedema in the treated group

Statistical Analysis

Data are expressed as mean $\pm$ standard error of the mean (SEM). Statistical analysis was performed using GraphPad Prism software (version 10.4.2). One-way analysis of variance (ANOVA) was used to evaluate differences among groups, followed by Tukey's post hoc test for multiple comparisons. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Acute Oral Toxicity Results

The acute oral toxicity studies showed no mortality or observable signs of toxicity, including nervousness, weakness, rashes, or abnormal behaviour, at administered doses up to 5000 mg/kg body weight in both phases of the study.

Table 1: Acute Toxicity Profile of *Hunteria umbellata* Seed (HUS)

Group	Dosage of Extract (mg/kg)	Mortality Rate
Phase 1		
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
Phase 2		
Group 1	1600	0/3
Group 2	2600	0/3
Group 3	5000	0/3

Analgesic activity

Acetic acid-induced writhing

Administration of *Hunteria umbellata* seed extract (HUS) produced dose-dependent but variable effects. The 500 mg/kg dose of the extract produced 49.37% inhibition at 5 min compared with control (15.80 ± 4.51 writhes). This effect reduced at later intervals, yielding negative inhibition values at 10 and 15 min. The 1000 mg/kg dose demonstrated a more sustained response, with peak inhibition of 57.79% at 30 min (9.20 ± 2.58 writhes vs. 21.80 ± 4.91 in control; $p < 0.05$). In contrast, the 1500 mg/kg dose produced inconsistent effects. Aspirin (100 mg/kg), used as the reference drug, produced marked early inhibition (84.81% at 5 min), but its effect declined at later intervals (Figures 1 and 2).

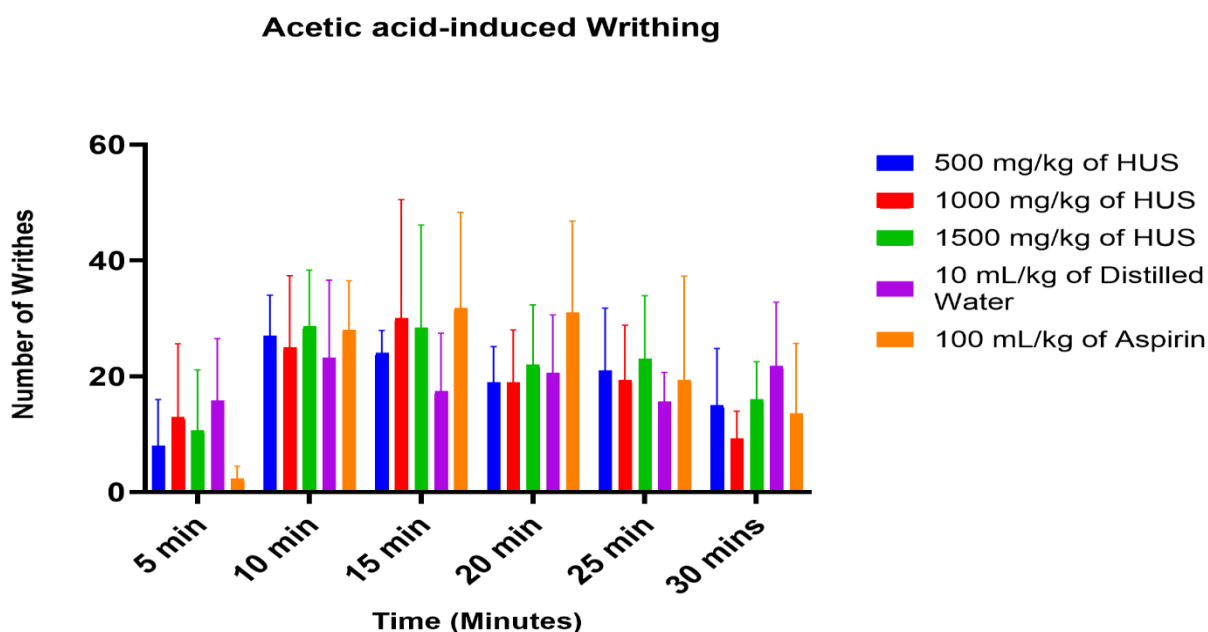


Figure 1: Acetic acid-induced writhing response in mice over time following treatment with *Hunteria umbellata* seed extract (HUS), distilled water, and aspirin. Data are expressed as mean $\pm$ SEM; $n = 5$; $p < 0.05$

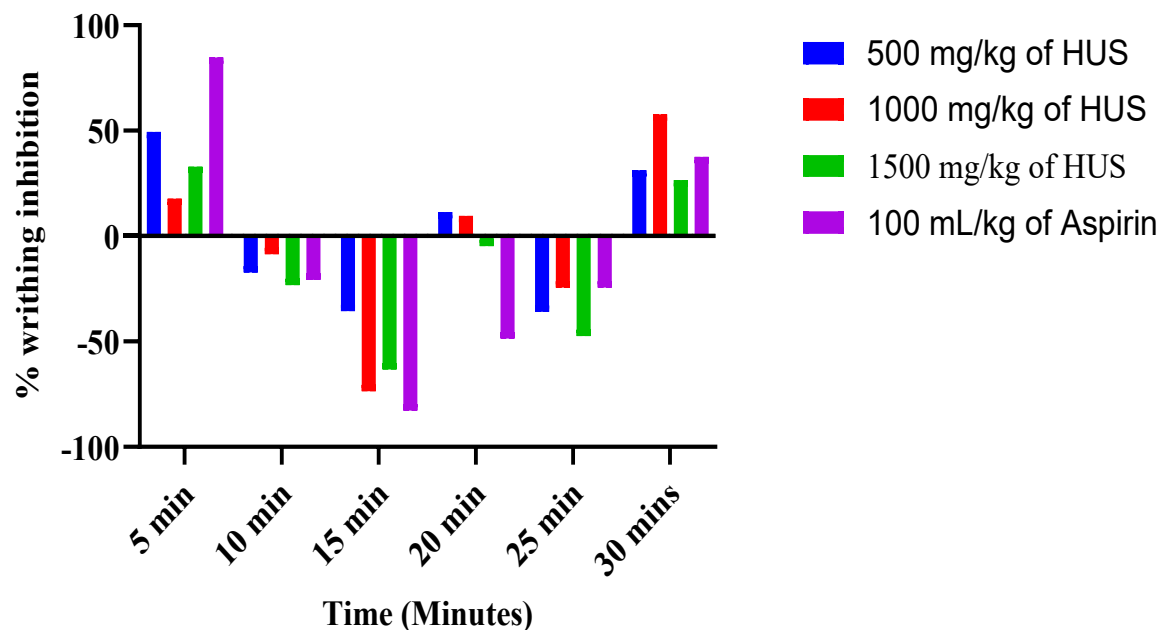


Figure 2: Percentage inhibition of Ethanolic extract of *Hunteria umbellata* Seed (HUS) on acetic acid-induced writhing in mice. Data are expressed as mean $\pm$ SEM; $n = 5$; $p < 0.05$.

Formalin-induced paw licking

At 500 mg/kg, HUS extract induced transient early-phase hyperalgesia (-38.59% inhibition at 5 min) but significantly attenuated late-phase nociceptive responses, achieving 69.72% inhibition at 30 min ($p < 0.05$). The 1000 mg/kg dose elicited the most consistent analgesic response, showing 76.15% inhibition at 30 min and a marked reduction in paw-licking duration (5.20 ± 3.76 min vs 21.80 ± 2.63 min in control; $p < 0.005$). In contrast, the 1500 mg/kg dose produced less consistent effects. Aspirin, used as a reference standard, reduced nociceptive responses in both phases, with peak inhibition of 43.55% at 15 min. as shown in Figures 3 and 4.

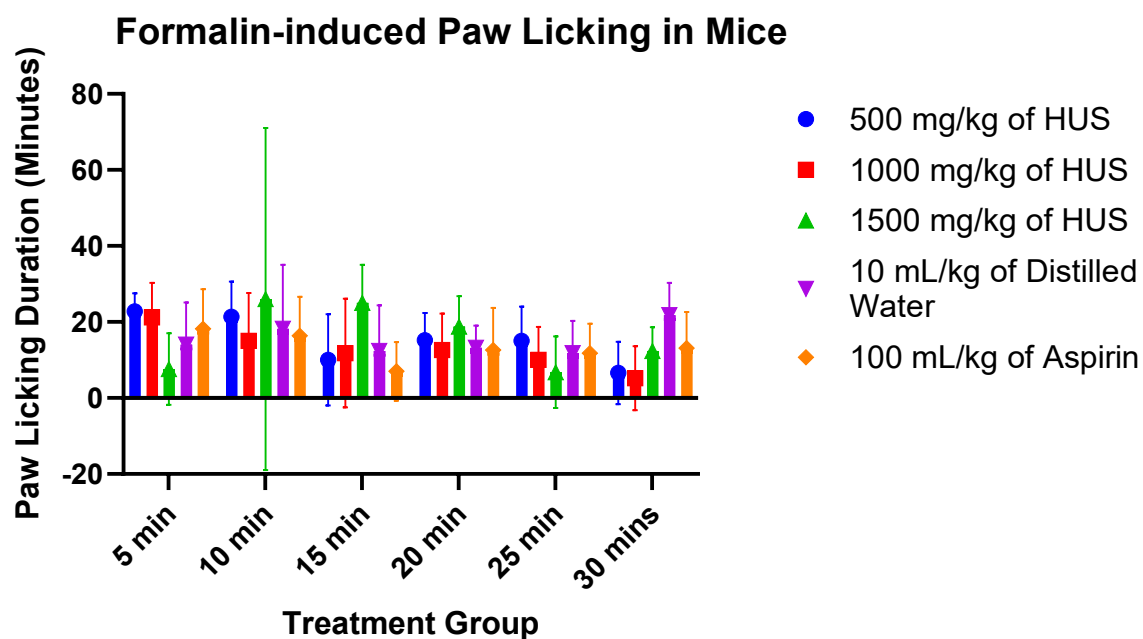


Figure 3: Effects of *Hunteria umbellata* Seed (HUS) extract on Formalin-Induced Paw Licking. Data are expressed as mean $\pm$ SEM; $n = 5$; $p < 0.05$

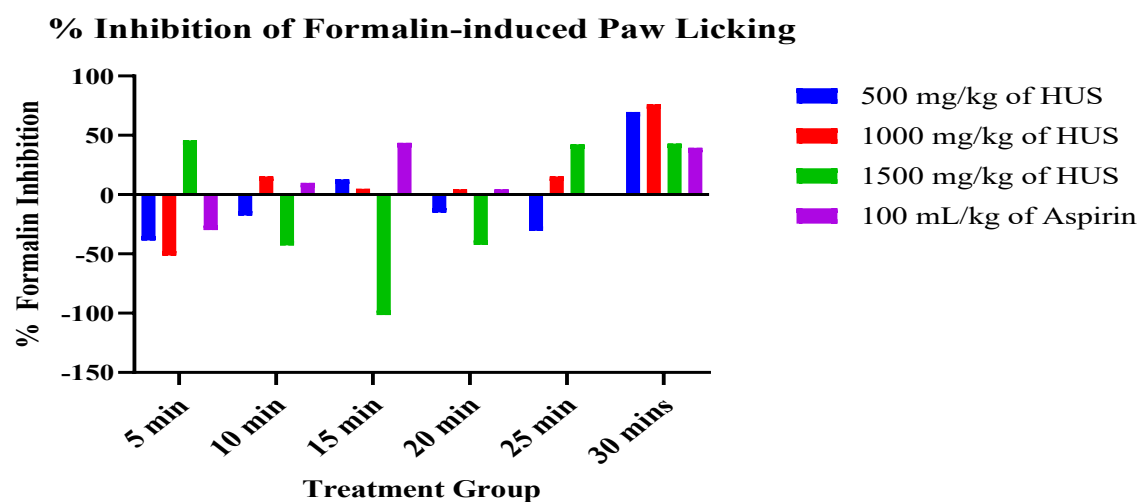


Figure 4: Percentage inhibition of formalin-induced paw licking behaviour following *Hunteria umbellata* Seed (HUS) extract administration. Data are expressed as mean $\pm$ SEM; $n = 5$; $p < 0.05$

Hot-plate test

Analgesic activity was assessed 30 minutes post-administration. The 1500 mg/kg dose produced the most pronounced effect, with a mean reaction time of 35.6 ± 2.67 s ($p < 0.05$), corresponding to a 163.3% increase over baseline and exceeding the response of

the reference drug dexamethasone (31.6 ± 2.85 s; 144.95 %). Lower doses also elicited significant prolongation of latency, reflecting consistent analgesic activity as shown in Figures 5 & 6.

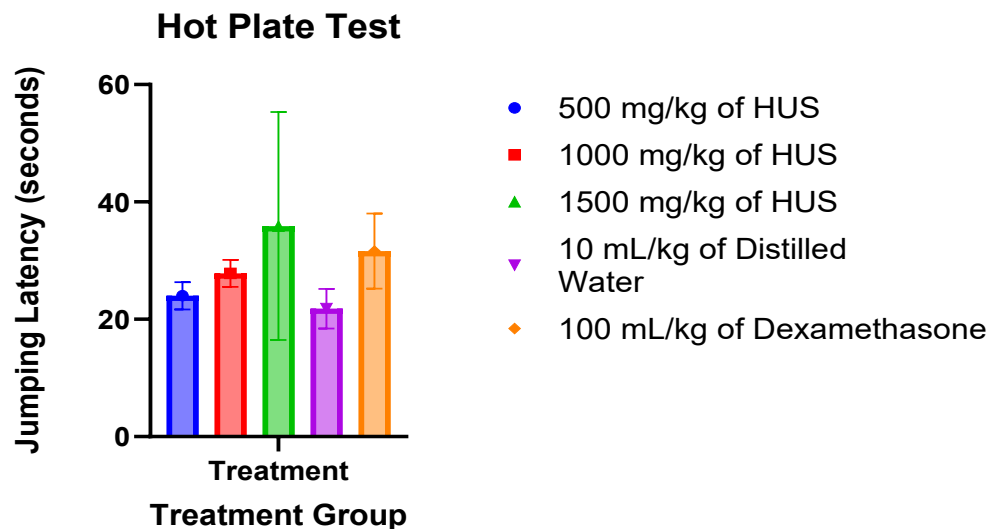


Figure 5: Effect of ethanolic seed extract of *Hunteria umbellata* Seed (HUS) on hot plate-induced pain in mice. Data are expressed as mean $\pm$ SEM; $n = 5$; $p < 0.05$

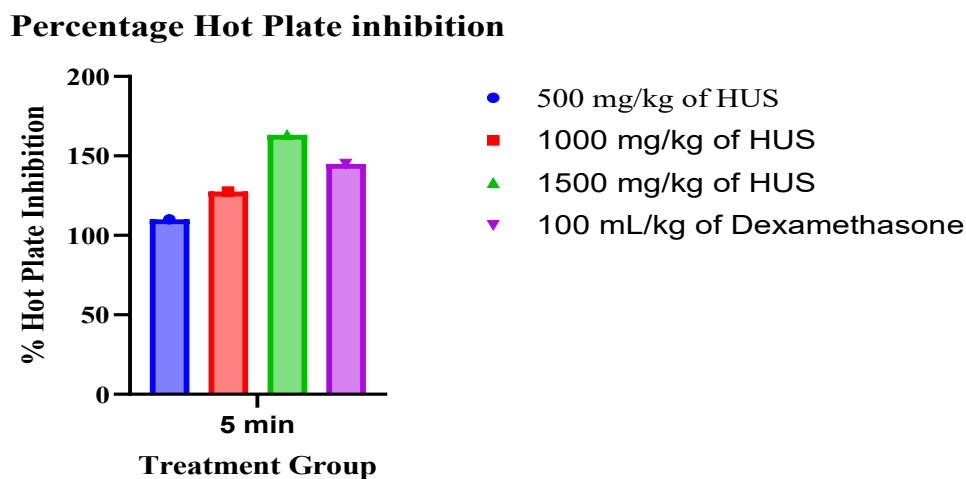


Figure 6: Percentage inhibition of ethanolic extract of *Hunteria umbellata* Seeds (HUS) on hot plate-induced pain in mice. Data are expressed as mean $\pm$ SEM; $n = 5$; $p < 0.05$

Anti-inflammatory activity

Carrageenan-induced paw oedema

At a dose of 500 mg/kg, *Hunteria umbellata* seed extract (HUS) reduced paw oedema thickness from 1.33 ± 0.00 mm at baseline to 0.52 ± 0.00 mm at 5 h (60.9% reduction; ($p < 0.05$)). The extract also produced 21.21% inhibition relative to the control group. A non-linear dose–response pattern was observed, with 1000 mg/kg and 1500 mg/kg groups demonstrating stronger late-phase anti-inflammatory effects (25.76% inhibition at 5 hours), surpassing the effect of aspirin (21.21%). The 1500 mg/kg group exhibited a biphasic response, characterized by transient elevations in oedema during the early inflammatory phase (0–3 h) before subsequent reduction in the late phase. Aspirin, used as the reference drug, showed modest inhibition throughout, with peak activity at 5 hours (21.21%) as shown in Figures 7 & 8.

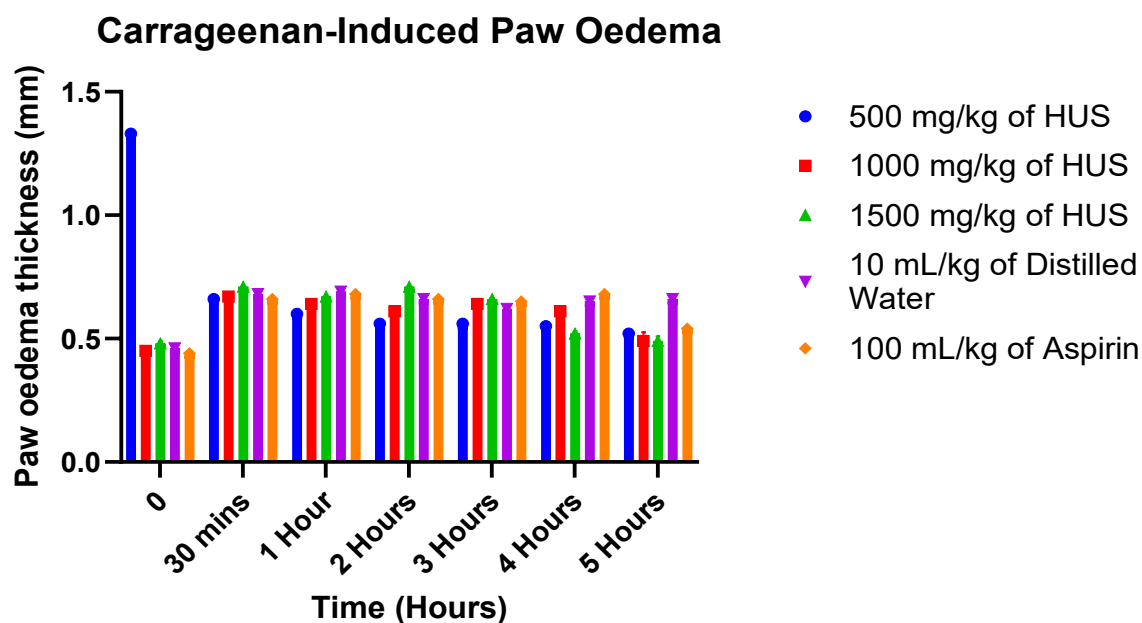


Figure 7: Effect of *Hunteria umbellata* seed (HUS) extract on carrageenan-induced paw oedema in mice. Data are expressed as mean $\pm$ SEM; $n = 5$; $p < 0.05$

Percentage Inhibition of carrageenan-induced oedema

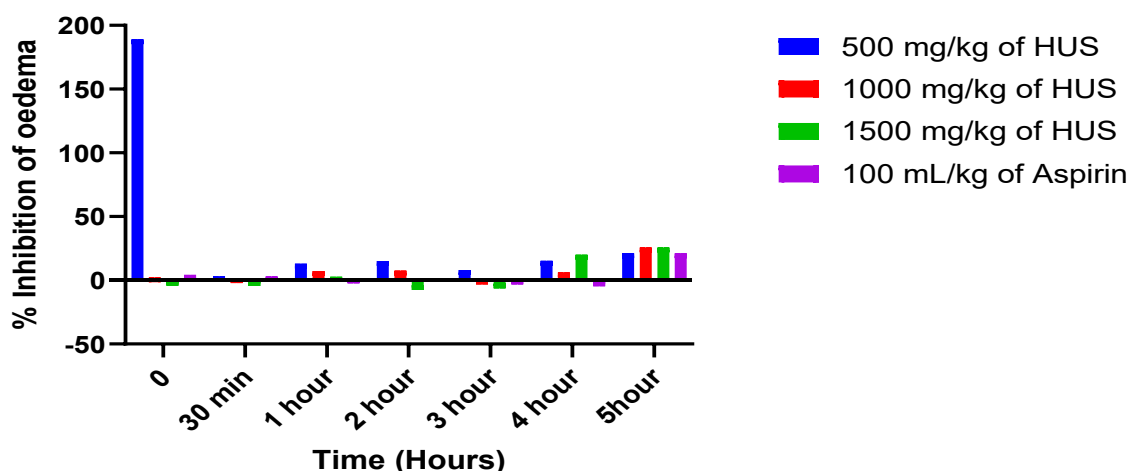


Figure 8: Percentage inhibition of ethanolic extract of *Hunteria umbellata* Seeds (HUS) on carrageenan-induced paw oedema in mice. Data are expressed as mean $\pm$ SEM; n = 5; p < 0.05

Egg albumin-induced paw oedema

At 5 hours post-induction, the 1500 mg/kg dose elicited the greatest inhibition of paw swelling (5.88%), approaching the effect of aspirin (7.84%). The 500 mg/kg group demonstrated moderate inhibition (3.92%), while the 1000 mg/kg group exhibited fluctuating responses across the observation period.

Egg albumin-induced Paw Oedema

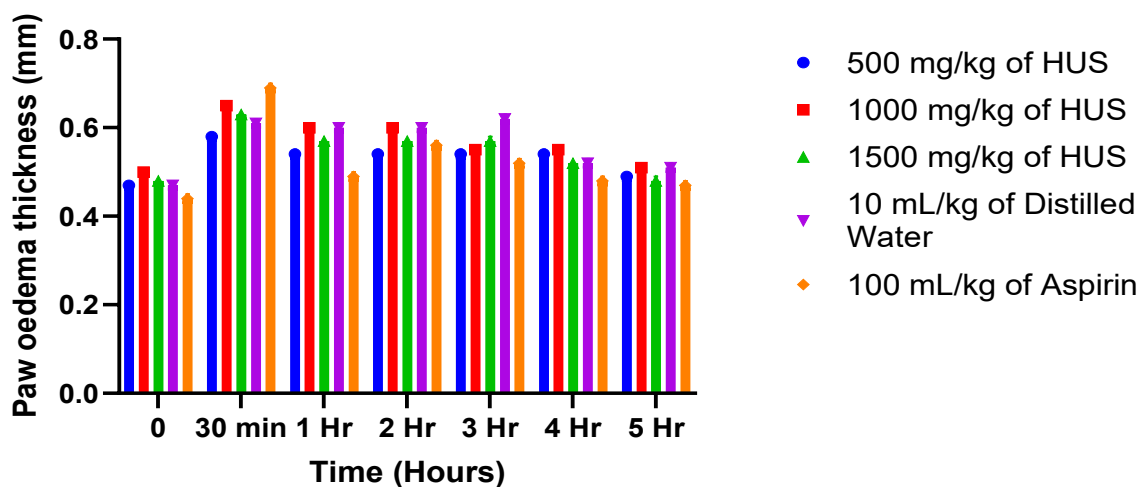


Figure 9: Effect of *Hunteria umbellata* seed (HUS) extract on Egg albumin-induced paw oedema in mice. Data are expressed as mean $\pm$ SEM; n = 5; p < 0.05

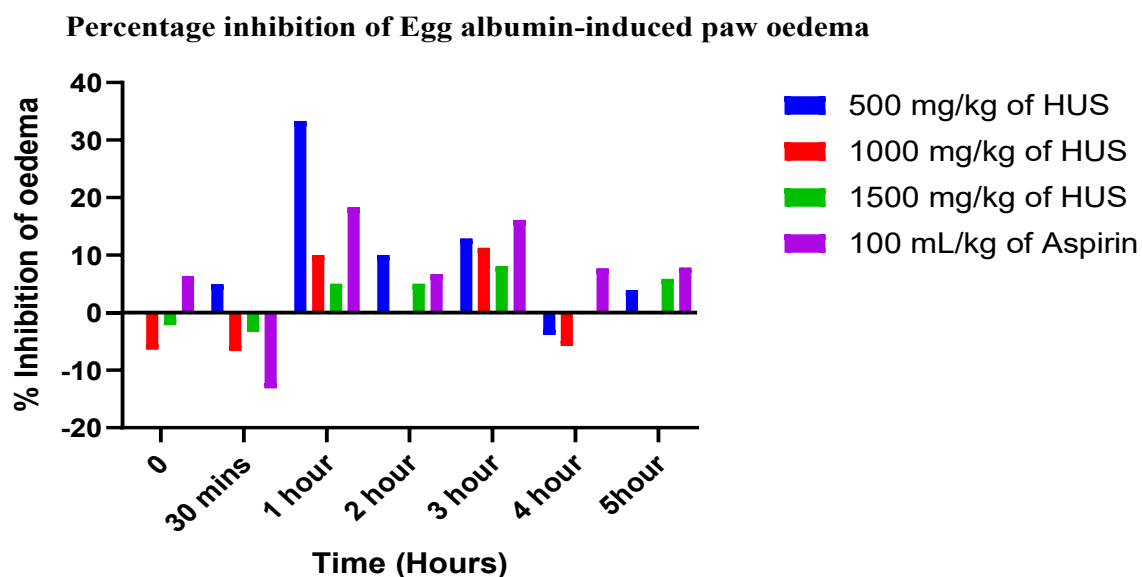


Figure 10: Percentage inhibition of ethanolic extract of *Hunteria umbellata* Seed (HUS) in egg albumin-induced paw oedema. Data are expressed as mean $\pm$ SEM; $n = 5$; $p < 0.05$

DISCUSSION

The present investigation demonstrated that the ethanolic seed extract of *Hunteria umbellata* exhibited measurable analgesic and anti-inflammatory effects, although the magnitude of anti-inflammatory activity varied across experimental models. Acute toxicity assessment revealed no mortality or obvious behavioural abnormalities at doses up to 5000 mg/kg body weight, suggesting that the extract possesses a relatively wide margin of safety according to OECD toxicity classification standards (OECD, 2001). Similar observations have been reported for other medicinal plant extracts with LD₅₀ values exceeding 5000 mg/kg in rodents (Akoh et al., 2024).

In the acetic acid-induced writhing assay, HUS extract reduced abdominal constrictions in treated mice, indicating peripheral antinociceptive activity. The analgesic effect observed in this model may be associated with inhibition of inflammatory mediators such as prostaglandins and other algogenic substances released following acetic acid administration (Meotti et al., 2006). Among the tested doses, the 1000 mg/kg dose produced the most consistent analgesic response, while the higher doses showed variability, reflecting the non-linear dose-response often associated with phytochemical-rich extracts (Jodynis-Liebert & Kujawska, 2020). The transient negative inhibition values observed at

certain doses may suggest temporary sensitization or a biphasic pharmacological response resulting from the complex interactions of bioactive phytochemicals present in the extract.

The formalin-induced paw licking test further confirmed the analgesic potential of the extract in both neurogenic and inflammatory phases of nociception. Suppression of the late inflammatory phase suggests possible interference with inflammatory mediator release and peripheral sensitization, whereas activity observed during the early phase may indicate involvement of central pain modulation pathways (Hunskar & Hole, 1987; Turnaturi et al., 2023). The observed activities may be associated with phytochemicals previously identified in the extract, including flavonoids, alkaloids, and saponins; however, the specific bioactive constituents responsible for these effects remain to be identified (Uka *et al.*, 2025).

Hot plate model demonstrated prolongation of reaction latency in extract-treated mice, further supporting centrally mediated analgesic activity. This increased latency time is associated with modulation of central nociceptive mechanisms involving opioid receptors and neurotransmitter pathways related to pain perception (Liktov-Busa et al., 2021). The pronounced analgesic response observed at higher doses may therefore suggest possible central nervous system involvement.

Findings from the carrageenan and egg albumin-induced models suggest that the extract interferes with multiple inflammatory mediators, including histamine, serotonin, kinins, and prostaglandins which are central to acute inflammation (Vinegar et al., 1969; Mohan, 2010). The observed pharmacological effects may be attributed to bioactive phytochemicals such as flavonoids, alkaloids, tannins, phenolic compounds, and saponins previously reported in *Hunteria umbellata* (Uka et al., 2025). These phytochemicals have previously been associated with antioxidant, anti-inflammatory, and analgesic activities in experimental studies (Wagner & Ulrich-Merzenich, 2009; Al Khayri et al., 2022). The observed pharmacological activities suggest that the seed extract of *Hunteria umbellata* may represent a promising natural source for the development of alternative therapeutic agents for pain and inflammatory disorders.

Conclusions

The ethanolic seed extract of *Hunteria umbellata* demonstrated potentials as a natural source of analgesic and anti-inflammatory compounds, exhibiting a significant safety margin at doses up to 5000 mg/kg. Our findings support the ethnomedicinal use of

the seed extract of *Hunteria umbellata* and highlight its promise as a candidate for future therapeutic development.

Recommendation

The ethanolic seed extract of *Hunteria umbellata* demonstrates promising analgesic and anti-inflammatory properties with a wide margin of safety. To advance its therapeutic potential, future studies should prioritize phytochemical isolation, mechanistic elucidation, and clinical validation, alongside formulation development and comparative studies with conventional pain and inflammation therapies

Contribution to Knowledge

This study contributes to knowledge by scientifically validating the analgesic and anti-inflammatory properties of ethanolic seed extract of *Hunteria umbellata*, establishing its safety profile, and providing mechanistic insights into its pharmacological actions. It advances ethnopharmacology by linking traditional use with experimental evidence, and lays the groundwork for future phytochemical isolation and clinical translation.

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