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Pharmacological Evidence for the Traditional Use of *Gmelina arborea* in Pain and Inflammation Management

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Abstract

Gmelina arborea is widely used in ethnomedicine for the treatment of pain and inflammatory disorders. This study evaluated the analgesic and anti-inflammatory activities of its ethanol leaf extract in murine models. Fresh leaves were extracted with 70% ethanol and screened for phytochemicals. Acute toxicity was determined using Lorke's method. Analgesic activity was assessed via acetic acid-induced writhing and thermal-induced pain tests, while anti-inflammatory activity was evaluated using formalin- and carrageenan-induced paw edema models. Diclofenac and pentazocine served as standard drugs. Data were analyzed using one-way ANOVA followed by Dunnett's post hoc test, with significance at $P < 0.05$. Phytochemical screening confirmed the presence of flavonoids, tannins, steroids, alkaloids, and saponins. The extract showed a high safety margin, with an LD_{50} of ~3808 mg/kg. In the writhing test, doses of 250, 500, and 1000 mg/kg inhibited abdominal constrictions by 20.00%, 33.33%, and 51.85%, respectively. In the thermal pain model, 500 and 1000 mg/kg significantly increased reaction latency, indicating central analgesic activity. In the formalin model, all doses reduced paw swelling, with higher doses showing stronger effects. In the carrageenan model, 500 and 1000 mg/kg significantly reduced edema at 60 min. Ethanol leaf extract of *G. arborea* possesses dose-dependent peripheral and central analgesic activities and moderate anti-inflammatory effects, supporting its traditional use and potential development as a plant-based therapeutic for pain and inflammation.

Keywords: *Gmelina arborea*, analgesic activity, anti-inflammatory activity, phytochemicals, murine models.

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Introduction

Pain is a multifaceted and complex biological experience that integrates both physiological and psychological responses to noxious stimuli. It serves as a crucial protective mechanism, prompting individuals to withdraw from harmful situations to prevent further tissue damage (Liu and Kelliher, 2022). Pain perception, however, is highly subjective and modulated by various factors, including psychological state, cultural background, cognitive processing, and genetic predisposition (Craig and MacKenzie, 2021). These variables account for individual differences in pain thresholds and tolerance, as observed in populations such as athletes who often exhibit greater pain resilience (Pettersen *et al*., 2020). Closely associated with pain is inflammation, a vital component of the body's innate immune response to harmful stimuli such as pathogens, physical injury, or irritants. This response involves a coordinated action of immune cells, vascular systems, and molecular mediators aimed at eliminating the offending agents, clearing damaged tissues, and initiating repair (Stone *et al*., 2025). Although protective, inflammation and pain can become chronic and contribute significantly to disease burden and reduced quality of life (Chavda *et al*., 2024).

Pharmacologic management of pain and inflammation commonly involves non-steroidal anti-inflammatory drugs (NSAIDs)**,** such as aspirin, ibuprofen, and naproxen, which exert both analgesic and anti-inflammatory effects (Alorfi, 2023). However, the widespread use of synthetic NSAIDs is limited by adverse effects, including gastrointestinal complications like peptic ulcer disease (Bindu *et al.,* 2020). These limitations underscore the urgent need for safer, more effective alternatives.

Medicinal plants represent a promising source of novel therapeutic agents. *Gmelina arborea***,** commonly known as beechwood, is one such plant with widespread traditional usage and a diverse pharmacological profile (Suneetha *et al.,* 2023). Indigenous to South and Southeast Asia and widely used in Ayurveda, Unani, and African traditional medicine systems, *G. arborea* has been employed for the treatment of pain, inflammation, fever, diabetes, aphrodisiac, piles, digestive disorders, respiratory conditions, liver diseases, heart diseases, and neurological ailments (Warrier *et al*., 2021; Wadasinghe *et al*., 2023). Phytochemical investigations have revealed that *G. arborea* contains a broad spectrum of bioactive constituents, including flavonoids, alkaloids, saponins, tannins, and phenolics known to possess antioxidant, antimicrobial, analgesic, and anti-inflammatory properties, reinforcing the plant's therapeutic potential (Warrier *et al*., 2021).

The exploration of such medicinal plants is particularly critical in rural and underserved areas where access to modern healthcare is limited, and traditional medicine remains a primary source of treatment (Tahir *et al*., 2023). In light of increasing drug resistance and side effects of conventional drugs, natural products offer a sustainable and culturally relevant approach to drug discovery. This study aims to investigate the analgesic and anti-inflammatory effects of ethanol leaf extract of *G. arborea* in mice.

Methodology

Plant Material Collection and Authentication

Fresh leaves of *Gmelina arborea* were collected from Gadau village in Itas-Gadau Local Government Area, Bauchi State, Nigeria. The plant was authenticated and identified by a taxonomist at the Herbarium Unit, Department of Biological Sciences, Bauchi State University, Gadau, where a voucher specimen was deposited for reference.

Experimental Animals

A total of 66 healthy albino mice (8–25 g) were procured from the Animal Facility of the Department of Pharmacology, Sa'adu Zungur University, Bauchi State. The animals were housed under standard laboratory conditions (12-hour light/dark cycle, room temperature 22–25°C), with access to standard feed and clean water ad libitum. All experimental procedures were approved by the Faculty of Basic Medical Sciences Research and Ethics Committee (FBMSREC) with approval letter number of BASUG/FBMS/REC/VOL.08/01048.

Equipment and Apparatus

The following materials and equipment were used: gloves, weighing balance, insulin syringes, oral gavage, animal cages, permanent markers, face masks, mortar and pestle, measuring cylinders, sample bottles, magnetic stirrer, spatulas, beakers, stopwatch, and filter paper.

Preparation of Plant Extract

The leaves of *Gmelina arborea* were washed with clean water, air-dried under shade, and ground into a fine powder using a mortar and pestle. A 100 g portion of the powder was subjected to cold maceration in 70% ethanol for 72 hours. The mixture was filtered using Whatman No. 1 filter paper, and the filtrate was evaporated to dryness at 45–50°C

using a water bath. The dried extract was stored in a clean, airtight container until use.

Phytochemical Screening

Qualitative phytochemical analysis of the extract was conducted using standard procedures to detect the presence of major secondary metabolites such as alkaloids, flavonoids, tannins, saponins, and phenolic compounds (Evans *et al*., 2002).

Acute Toxicity Study

The acute toxicity of the ethanol extract was evaluated following the method described by Lorke (1983), using a total of 13 mice. In the first phase, mice were divided into three groups (n=3), and each group received intraperitoneal (IP) doses of 10, 100, and 1000 mg/kg, respectively. In the second phase, based on the results of phase one, three additional mice received higher doses of 1600, 2900, and 5000 mg/kg. The median lethal dose (LD_{50}) was calculated as the geometric mean of the highest non-lethal dose and the lowest lethal dose.

Acetic Acid-Induced Writhing Test

The analgesic activity of the extract was assessed using the acetic acid-induced writhing model as described by Fontenele *et al*. (1996). Fifteen mice were randomly divided into five groups (n=3):

Group 1 (Negative Control) received normal saline (5 mL/kg, ip)

Groups 2–4 (Test Groups) received the extract at doses of 1000, 500, and 200 mg/kg (ip), respectively

Group 5 (Positive Control) received diclofenac sodium (10 mg/kg, ip)

Thirty minutes post-treatment, all animals were administered 0.06% acetic acid (10 mL/kg, IP). Five minutes after injection, the number of abdominal constrictions (writhes) was counted for each mouse over a 10-minute observation period. The percentage inhibition of writhing was calculated as:

$$\%\ Inhibition = \frac{Ncontrol - Ntest}{Nc} \times 100$$

Where Ncontrol is the mean number of writhes in the control group, and Ntest is the mean number in the treatment group.

Hot Plate thermal pain test

The hot plate test was conducted as described by Gandigawad *et al.* (2018). Fifteen mice were randomly divided into five groups (n=3). Group 1, 2 3 and 4 were treated as described above, while Group 5 was administered Pentazocine 10 mg/kg. Each mouse was placed on the hot plate at a temperature of 45-50°C at intervals of 0, 15, 30, and 60 minutes post-treatment, and the reaction time to pain described as paw licking or jumping was recorded in seconds.

Effect of the extract on Formalin--induced Paw Edema

The anti-inflammatory activity of the extract was evaluated using the formalin-induced paw edema model in mice as described by Hassan *et al*., (2025). Group 1, 2 3 and 4 were treated as described in acetic acid-induced pain, while Group 5 was administered Diclofenac 25 mg/kg. Paw edema was induced by injecting 0.1 mL of 1% formalin into the sub plantar region of the right hind paw of each mouse. The extract or standard drug was administered 1 hour before formalin injection. Paw thickness was measured using a veneer caliper at 0,15,30 and 60 minutes post-formalin injection.

Effect of the extract on Carrageenan-Induced Paw Edema

The anti-inflammatory activity of the extract was evaluated using the formalin-induced paw edema model in mice as described by Kaur et al. (2018). Group 1, 2 3 and 4 were treated as described in acetic acid-induced pain, while Group 5 was administered Diclofenac 25 mg/kg. Paw edema was induced by administering 0.05 mL of freshly prepared 1% Carrageenan solution subcutaneously into the right hind paw of each mouse, and the paw volumes were measured using a digital vernier caliper at 0, 15, 30 and 60 minutes after carrageenan injection.

Statistical Analysis

Data were analyzed using GraphPad Prism version 9. Results were expressed as mean ± standard error of the mean (SEM). One-way analysis of variance (ANOVA) followed by Dunnett's post hoc test was used to compare the treatment groups with the control. Differences were considered statistically significant at $P < 0.05$.

Results

Percentage Yield of the Extract

Extraction of approximately 397.8 g of the powdered leaves of *G. arborea* with 70% ethanol yielded 14.4 g of a dark green, semi-solid extract. This corresponds to a percentage yield of 3.62%.

Phytochemical Constituents of the Extract

Preliminary qualitative phytochemical screening of the ethanol extract revealed the presence of flavonoids, tannins, steroids, alkaloids, and saponins **(**Table 1**).**

Acute toxicity test

No mortality was occurred at doses up to 2900 mg/kg, however there was a mortality at 5000 mg/kg. Thus, the estimated LD_{50} of the ethanol leaf extract of *G. arborea* was approximately 3808 mg/kg.

Effect of the extract on Acetic Acid-induced Writhing Test

The extract demonstrated a dose-dependent reduction in the number of abdominal writhing induced by acetic acid in mice. The untreated control group (normal saline, 10 mL/kg) exhibited a mean writhing count of 45.00 ± 2.89. Administration of the extract at 1000 mg/kg significantly ($p < 0.05$) reduced writhing to 21.67 ± 1.76, corresponding to a 51.85%. The effect of the extract was dose-dependent (Figure 1).

Effect of the extract on hot plate-induced thermal pain

The extract increased pain reaction latency in the thermal-induced pain test in mice, suggesting analgesic activity (Table 2). At 500 and 1000 mg/kg, the extract produced significant increases in pain latency compared to normal saline ($P < 0.05$), with the highest effect observed at 60 min

Anti-inflammatory effect of the extract on formalin-induced edema in mice

The ethanol leaf extract of *G. arborea* produced a dose-dependent reduction ($P < 0.05$) in paw edema in formalin-induced inflammation in mice (Table 3).

Anti-inflammatory effect of the extract on caageenan-induced edema in mice

The extract at 500 and 1000 mg/kg, significantly inhibited paw swelling at 60 min ($P < 0.05$) compared to the normal saline group (Table 4. The 250 mg/kg dose evoked minimal activity, with only a transient increase at 15 min.

Table 1. Phytochemical constituents of ethanol leaf extract of *G. Arborea*

Phytochemical Test	Inference
Flavonoids	+
Tannins	+
Steroids	+
Alkaloids	+
Saponins	+

(+) = Present; (−) = Absent

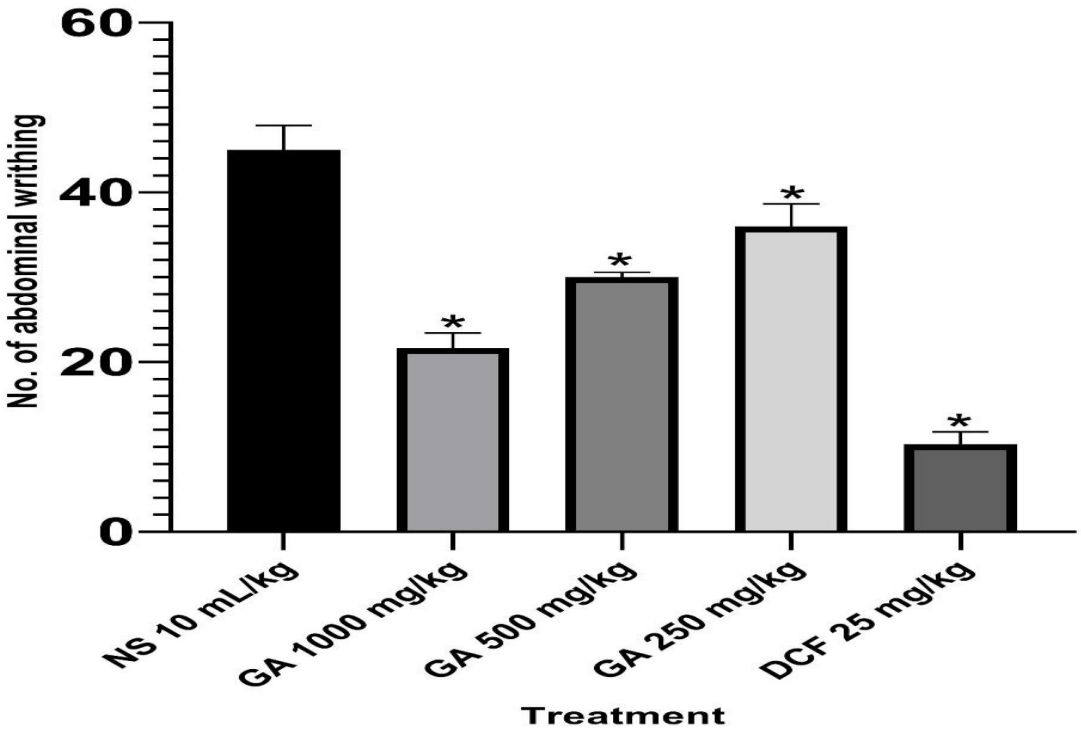

Figure 1. Effect of ethanol extract of *G. arborea* (GA) on acetic acid-induced writhing test in mice. NS= Normal saline; DCF = Diclofenac. * $P < 0.05$ in comparison with the normal saline control group (one-way ANOVA followed by Dunnet post hoc test).

Table 2. Effects of ethanol leaf extract of *G. arborea* on thermal-induced pain in mice

Treatment (mg/kg)	0 min	15 min	30 min	60 min
Normal saline 10 mL/kg	0.81 ± 0.04	1.81 ± 0.01	1.86 ± 0.11	1.48 ± 0.18
G. arborea 250	0.63 ± 0.01	1.82 ± 0.04	1.83 ± 0.06	2.27 ± 0.20
G. arborea 500	1.59 ± 0.08*	1.24 ± 0.18	2.64 ± 0.06*	3.21 ± 0.05*
G. arborea 1000	2.87 ± 0.25*	2.34 ± 0.03	2.47 ± 0.02*	3.82 ± 0.12*
Pentazocine 10	4.44 ± 0.09*	2.62 ± 0.28*	5.93 ± 0.02*	5.28 ± 0.44*

Data are expressed as Mean ± Standard Error of Mean (SEM), n=3. Analysis was performed using one-way ANOVA, followed by Dunnett's post hoc test, with the normal saline group as the reference. * $P < 0.05$.

Table 3. Effects of ethanol leaf extract of *G. arborea* on formalin-induced paw edema in mice

Treatment (mg/kg)	0 min	15 min	30 min	60 min
Normal saline 10 mL/kg	3.27 ± 0.19	2.92 ± 0.17	2.68 ± 0.20	2.41 ± 0.24
G. arborea 250	3.31 ± 0.07	3.12 ± 0.06	2.76 ± 0.18	2.10 ± 0.44
G. arborea 500	2.56 ± 0.27*	3.34 ± 0.03	2.57 ± 0.15	2.10 ± 0.04
G. arborea 1000	3.09 ± 0.21	2.95 ± 0.03	2.83 ± 0.05	2.08 ± 0.04
Diclofenac 25	3.35 ± 0.15	2.81 ± 0.07	1.92 ± 0.19*	1.36 ± 0.13*

Data are expressed as Mean ± Standard Error of Mean (SEM), n=3. Analysis was performed using one-way ANOVA, followed by Dunnett's post hoc test, with the normal saline group as the reference. * $P < 0.05$ (significantly different from Control).

Table 4. Effects of ethanol leaf extract of *G. arborea* on carrageenan-induced edema in mice

Treatment (mg/kg)	0 min	15 min	30 min	60 min
Normal saline 5 mL/kg	2.96 ± 0.24	2.75 ± 0.16	2.51 ± 0.15	2.66 ± 0.13
G. arborea 250	3.15 ± 0.22	3.18 ± 0.04*	2.73 ± 0.16	2.51 ± 0.05
G. arborea 500	2.61 ± 0.13	3.38 ± 0.05*	2.81 ± 0.05	2.07 ± 0.03*
G. arborea 1000	3.06 ± 0.20	2.91 ± 0.03	2.84 ± 0.04	2.09 ± 0.04*
Diclofenac 25	2.78 ± 0.14	2.68 ± 0.05	2.16 ± 0.01	1.69 ± 0.12*

Data are expressed as Mean ± Standard Error of Mean (SEM), n=3. Analysis was performed using one-way ANOVA, followed by Dunnett's post hoc test, with the normal saline group as the reference. * $P < 0.05$.

Discussion

The findings of this study provide insights into the safety profile, analgesic and anti-inflammatory potential of the ethanol leaf extract of *Gmelina arborea*. Phytochemical analysis of the extract revealed compounds with documented pharmacological activities, particularly in the management of pain and inflammation (Santiago *et al*., 2021, Al-Khayri *et al*., 2022; Wijesekara *et al*., 2024).

Flavonoids, in particular, such as luteolin and apigenin, are known inhibitors of cyclooxygenase (COX) and lipoxygenase (LOX) enzymes, thereby reducing prostaglandin synthesis and modulating nociceptive responses (Idris *et al*., 2022). The presence of alkaloids and saponins may also contribute to analgesic effects via central nervous system modulation and stabilization of cell membranes, respectively (Heinrich *et al*., 2021; Wijesekara

et al., 2024). These bioactive constituents likely act synergistically to produce the observed pharmacological effects.

The acute toxicity study revealed that the extract is relatively safe at doses up to 2900 mg/kg, with the estimated median lethal dose calculated to be approximately 3808 mg/kg. No mortality or signs of acute toxicity were observed at lower doses during the 24-hour observation period. According to Lorke's (1983) classification, substances with an LD_{50} greater than 3000 mg/kg are considered practically non-toxic, indicating that *G. arborea* ethanol extract has a high safety margin (Hodge and Sterner, 1949). This supports its traditional use in ethnomedicine and underscores its potential for further development as a therapeutic agent.

The antinociceptive activity assessed through the acetic acid-induced writhing test demonstrated a dose-dependent analgesic effect**.** The extract significantly reduced the number of writhes at all doses tested, with the highest dose (1000 mg/kg) producing 51.85% inhibition of writhing. Although diclofenac (10 mg/kg) achieved a higher inhibition of 77.04%, the substantial reduction in pain behaviors by *G. arborea* extract especially at 1000 mg/kg indicates meaningful peripheral analgesic activity.
Furthermore, the ethanol leaf extract of *G. arborea* also shows noble anti-inflammatory activity in formalin-induced paw edema in mice. This dose-dependent anti-inflammatory activity corresponds to previous findings in similar models (Gandigawad *et al*., 2018; Kaur *et al.,* 2018).

The carrageenan-induced paw edema model is widely utilized to assess both neurogenic (early phase) and inflammatory (late phase) components of inflammation. The low dose of the extract was insufficient to inhibit early-phase inflammatory mediators and might have transiently exacerbated vascular permeability. There was a significant increase in paw volume at 15 minutes (3.38 ± 0.05; $P < 0.01$), possibly due to an initial irritant response or delay in the pharmacodynamic onset of active phytochemicals. The notable reduction in edema at the later phase indicated potent suppression of the inflammatory phase. This result parallels the observations of Kaur *et al*. (2018), who found that methanol bark extract of *G. arborea* at 500 mg/kg significantly inhibited carrageenan-induced inflammation in rats, likely due to flavonoid constituents such as GM-01. The more rapid and sustained anti-inflammatory effect at higher doses, suggests better bioavailability or synergy among bioactive constituents.

Theses pharmacological effects can be traced to the phytochemical profile characteristic of Gmelina species. These include flavonoids, iridoid glycosides, lignans, tannins, phenolic acids, saponins, and terpenoids (Warrier *et al*., 2021). Flavonoids such as luteolin, quercetin, and apigenin derivatives inhibit COX and LOX, reducing prostaglandin synthesis and inflammatory pain (Mohammed, 2022). Iridoid glycosides (e.g., gmelinosides A–L) may mediate central analgesia via modulation of opioid receptors and monoaminergic pathways, while lignans like gummadiol could further support central nervous system analgesia (Kaur *et al*., 2018). Kaushik et al. (2024) demonstrated via in silico docking and in vivo models that these compounds exhibit strong binding affinity to Janus kinase 3 (JAK3), a key player in pro-inflammatory cytokine signaling, and significantly reduced arthritic and inflammatory symptoms in rat models (Kaushik *et al*., 2024). Tannins and saponins contribute by stabilizing cell membranes and inhibiting inflammatory mediator release, complementing peripheral analgesic effects (Hassan *et al*., 2025). Additionally, phenolic compounds and terpenoids bolster antioxidative defenses, mitigating oxidative stress implicated in chronic inflammation and nociception (Dalhatu *et al*., 2024). Prior ethnopharmacological use of *G. arborea* for pain and inflammation disorders, including arthritis and rhinitis, further validates these experimental outcomes (Anthony *et al*., 2012; Kulkarni *et al*., 2013).

Conclusion

Ethanol leaf extract of *G. arborea* possesses dose-dependent peripheral and central analgesic activities and moderate anti-inflammatory effects, supporting its traditional use and potential development as a plant-based therapeutic for pain and inflammation.

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Conflicts of interest

The author declares that there is no conflict of interest.

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