Original Article

Pharmacological Assessment of *Indigofera hochstetteri*: Anti-inflammatory and Analgesic Potential

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ABSTRACT

Background: Inflammation and pain are essential physiological responses that protect the body against harmful stimuli. However, when chronic, they can result in severe health problems and disability. The available treatment options have significant limitations, necessitating the search for safer alternatives.

Objectives: This study aimed to evaluate the anti-inflammatory and analgesic potential of the methanol leaf extract of *Indigofera hochstetteri*.

Methods: The methanol extract of *I. hochstetteri* was subjected to phytochemical screening and acute toxicity assessment using Lorke's method. Anti-inflammatory activity was examined using formalin-induced paw edema and xylene-induced ear edema, while acetic acid-induced writhing and hot plate tests were employed to assess analgesic activity.

Results: Phytochemical analysis indicated the presence of flavonoids, glycosides, terpenes, and saponins. Acute toxicity studies indicated a moderate toxicity level, with a median lethal dose of 2,154 mg/kg. The extract displayed statistically significant ($P<0.05$) anti-inflammatory effects in both the formalin and xylene models. Substantial analgesic activity was also observed in both the writhing and hot plate tests. The extract's efficacy was directly proportional to the dose, with higher doses (300 and 600 mg/kg) showing effectiveness similar to that of standard drugs.

Conclusion: These findings imply that *I. hochstetteri* has promising potential as a natural alternative for managing inflammation and pain.

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Introduction

Inflammation and pain are fundamental physiological responses that are critical components of the body's defense mechanism. Despite these protective roles, chronic pain can become pathological, often associated with depression, anxiety, and sleep disturbances [1]. Chronic inflammation, on the other hand, is a common underlying factor in many diseases, including cardiovascular diseases, cancer, autoimmune diseases, and metabolic disorders, substantially contributing to health burden [2].

Non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids are often employed in the treatment of inflammation, while NSAIDs and opioids are used for pain management. However, prolonged use of NSAIDs can lead to gastrointestinal adverse effects, increased risk of cardiovascular events, and renal impairment, especially in patients with pre-existing kidney conditions, limiting their safety for chronic use [3]. The most pressing issues with opioid use include a wide range of adverse effects, potential for addiction and abuse, tolerance, and withdrawal symptoms, making them a less-than-ideal solution for chronic pain management [4, 5]. Long-term use of corticosteroids, which are potent anti-inflammatory agents, includes weight gain, osteoporosis, osteonecrosis, diabetes, hypertension, myopathy, cataracts, glaucoma, immunosuppression, psychiatric disturbances, gastrointestinal, endocrine, and dermatological adverse effects [6].

The search for better and safer alternatives to conventional treatments for inflammation and pain has led to renewed interest in plant-based therapies. Plants have been used for medicinal purposes for centuries, and modern research has increasingly validated their efficacy and safety [7]. Therefore, as studies continue to uncover the therapeutic potential of various plants, this study aimed to evaluate the anti-inflammatory and analgesic potential of *Indigofera hochstetteri*.

I. hochstetteri is a plant species belonging to the *Indigofera* genus within the family Fabaceae, the third largest family of land plants in terms of species, with about 19000 known species and 751 genera [8]. *I. hochstetteri* is found in various regions, including Kenya, Tanzania, Uganda, Chad, Congo, Djibouti, Eritrea, Ethiopia, Socotra, Somalia, Sudan, Algeria, Egypt, Angola, Zambia, Benin, Burkina, Mali, Mauritania, Nigeria, Niger, Senegal, Cameroon, Zaire, Oman, Saudi Arabia, Yemen, India, and Pakistan [9, 10]. Different parts of plants in the *Indigofera* genus have been widely employed in the treatment of pain and inflammatory conditions, swellings, arthritis, wounds, skin diseases, gastrointestinal disorders, respiratory diseases, malaria, menstrual cramps, dysmenorrhea, infertility, uterine fibroids, premature ejaculation, diabetes, epilepsy, liver diseases, eczema, scabies, abscess, leprosy, hepatitis, worms, gonorrhea, HIV-related symptoms, diphtheria, tuberculosis, snake bites, epistaxis, and jaundice [11].

Pharmacological studies of crude extracts and purified fractions of different *Indigofera* species have reported various properties, including analgesic, anti-arthritic, antidiabetic and antidyslipidemic, antidiarrheal, anti-inflammatory, antipyretic, antioxidant, antifungal, antitrypanosomal, antiplasmodial, antimicrobial, anthelmintic, antifilarial, antiulcerogenic and gastroprotective, antiviral, hepatoprotective, hypotensive, immunomodulatory, insecticidal, anticancer, anticonvulsant, neuroprotective, nephroprotective, reproprotective, genoprotective, wound-healing, and skin rejuvenating activities [11-15]. Despite the widespread usage of these plants, *I. hochstetteri* remains largely unexplored, resulting in a paucity of data on its medicinal potential.

Materials and Methods

Experimental animals

Swiss albino mice and Wistar rats weighing 15-38 g and 150-200 g of either sex were obtained from the National Veterinary Research Institute, Vom, Plateau State. The animals were transported humanely and housed at the Animal House of the Department of Pharmacology, Bauchi State University, Gadau, Nigeria. The animals were fed with a standard pellet diet and water ad libitum. They were kept under standard conditions (12-hour light and 12-hour dark cycle) in a propylene cage at room temperature.

Plant collection and identification

The whole plant of *I. hochstetteri* was collected in July 2019 at Samaru village in Zaria local government area of Kaduna state. It was identified at the collection point and further authenticated at the Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria (voucher specimen number: 900153).

Preparation and extraction procedure

The *I. hochstetteri* plant was air-dried, reduced to a powder, labeled, and stored at room temperature for sub-

sequent use. A total of 4, 162 g of the powdered plant was extracted using 70% v/v methanol using the maceration method for 7 days. The extract was evaporated in vacuo using a rotary evaporator to yield a dark brown residue (396 g), subsequently referred to as the methanol plant extract.

Preliminary phytochemical screening

The qualitative phytochemical constituents of the methanol plant extract were screened using a standard protocol [16].

Acute toxicity studies

The acute toxicity profile of *I. hochstetteri* was evaluated intraperitoneally in mice as Lorke (1983) described [17]. Nine mice of either sex were divided into three groups containing three mice each in the first phase and administered 10, 100, and 1000 mg/kg of the extract. In the second phase, three animals received 1600, 2900, and 5000 mg/kg of the extract based on the outcomes of the phase one study. The median lethal dose was calculated using the Equation 1:

1. $LD_{50}=\sqrt{((\text{Minimal}) \text{ lethal dose} \times \text{Maximal nonlethal dose})}$

Anti-inflammatory study

1. Formalin-induced paw edema in mice

The chronic anti-inflammatory effect of the methanol extract of *I. hochstetteri* was evaluated using formalin-induced paw edema as described by Eddouks et al. (2012) [18]. Twenty-five mice were divided into five groups of five animals. Thirty minutes before formalin injection, animals in group one were administered 10 mL/kg normal saline, while group five received Indomethacin at 10 mg/kg. Groups two, three, and four were administered graded doses of the extract at 150, 300, and 600 mg/kg, respectively, after which inflammation was induced by sub-plantar injection of 20 mL of freshly prepared 2% formalin in the right hind paw. Paw thickness was measured using a digital caliper at 0, 30, 60, and 90 minutes, and increases in paw thickness were recorded.

2. Xylene-induced ear edema test in rats

The method described by Hosseinzadeh and Younesi (2002) [19] was used to assess the acute anti-inflammatory effect of *I. hochstetteri* in rats. The animals were divided into five groups consisting of five animals each. Groups one and five served as the negative and standard control groups, respectively. Animals in group one were administered 10 ml/kg of normal saline, while those in group five received dexamethasone at 10 mg/kg. Groups two, three, and four were given graded doses of the extract at 150, 300, and 600 mg/kg, respectively. Thirty minutes after the intraperitoneal injection of the extract, 0.03 mL of xylene was applied to the anterior and posterior surfaces of the right ear. The left ear was considered a control, and 15 minutes later, the animals were euthanized. Both ears were removed and weighed, and the increase in weight caused by the irritant was calculated by taking the difference between the weights of the untreated left and treated right ear sections.

Analgesic study

1. Acetic acid-induced writhing test

For the writhing test, twenty-five mice were randomly divided into five groups of five mice. Group one was treated with normal saline 10 mL/kg, groups two, three, and four were treated with graded doses of the extract at 150, 300, and 600 mg/kg, respectively, and group five was treated with piroxicam at 10 mg/kg. All drugs were administered intraperitoneally, and 30 minutes later, each mouse was injected intraperitoneally with 10 ml/kg of 0.6% acetic acid. The animals were placed in a transparent cage and observed for 10 minutes for abdominal writhing (constriction of the abdominal muscle and stretching of the hind and limb count). The percentage inhibition of abdominal writhing was calculated using the Equation 2:

2. $\%\ \text{Inhibition}=\frac{Nc-Nt}{Nc}\times 100$

Where Nc and Nt equal the number of abdominal writhings in the control and test groups, respectively.

2. Hot plate test

The central analgesic effect of the extract was evaluated using a hot plate test as described by [20]. Before the test, mice were placed on a hot plate maintained at a temperature of 50-55 ˚C, and only mice exhibiting an initial nociceptive response within the first 20 seconds were included in the study. Twenty-five mice were randomly grouped into five groups, n=5. Group one was treated with 10 ml/kg of normal saline, groups two, three, and four were treated intraperitoneally with 150, 300, and 600 mg/kg of the extract, respectively, while group five was treated with Pentazocine at 10 mg/kg. All drug administrations were done via the intraperitoneal route. Thirty minutes later, animals were placed on a hot plate

Table 1. Acute toxicity profile of the whole plant methanol extract of *I. hochstetteri*

Phases	Dose (mg/kg)	Mortality
Phase I	10	0/3
	100	0/3
	1000	0/3
Phase II	1600	0/1
	2900	1/1
	5000	1/1

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maintained at 50-55 ˚C, and the reaction time (latency) to pain response indicated through jumping or paw licking was recorded using a stopwatch at 0, 30, 60, and 90 minutes. A cut-off time of 20 s was set to reduce skin injury.

Statistical analysis

All data obtained were analyzed using one-way analysis of variance (ANOVA) followed by the Dunnet post hoc and expressed as MEAN±SEM, and percentages. Differences were considered significant at $P<0.05$. Analyses were conducted using SPSS software, version 22.

Results

Percentage yield and phytoconstituents of the extract

The extraction of approximately 4,162 kg of *I. hochstetteri*'s whole plants yielded 396 g of the methanol extract, corresponding to a yield of 9.5%. Qualitative phytochemical assessment of the extract detected the presence of flavonoids, glycosides, steroids/terpenes, and saponins, while tannins, alkaloids, and anthraquinones were absent.

Median lethal dose value (LD_{50})

In phase I, no mortality was observed in mice administered 10, 100, and 1000 mg/kg doses. However, in phase II, mortality was observed at higher doses: One mouse died at 2900 mg/kg, and another at 5000 mg/kg. The 1600 mg/kg dose did not result in mortality in the mice tested (Table 1). The calculated lethal dose (LD_{50}) of the extract was approximately 2,200 mg/kg.

Methanol extract of *I. hochstetteri* exhibits anti-inflammatory action

1. Formalin-induced paw edema in mice

The whole plant methanol extract of *I. hochstetteri* at 300 and 600 mg/kg doses significantly reduced formalin-induced paw edema in mice at 30, 60, and 90 minutes. The extract at the 150 mg/kg dose only produced a significant reduction in paw edema at 90 minutes (Table 2).

Table 2. Effect of methanol extract of *I. hochstetteri* on formalin-induced paw edema in mice (n=5)

Treatment	Dose (mg/kg)	Mean±SEM			
		0 min (s)	30 min (s)	60 min (s)	90 min (s)
Normal saline	10 mL/kg	4.08±0.2	4.39±0.21	4.57±0.17	4.68±0.16
I. hochstetteri	150	4.45±0.11	4.43±0.1	4.32±0.12	4.21±0.12*
I. hochstetteri	300	4.73±0.02	4.59±0.04*	4.39±0.04*	4.12±0.02*
I. hochstetteri	600	4.59±0.1	4.35±0.07*	4.19±0.04*	4.09±0.05*
Indomethacin	10	4.35±0.14	3.97±0.18*	3.70±0.15*	3.45±0.11*

*Significant difference compared to the normal saline control group (one-way ANOVA followed by Dunnett post hoc test) ($P<0.05$).

Table 3. Effect of methanol extract of *I. hochstetteri* on pain latency in hot plate test in mice (n=5)

Treatment	Dose (mg/kg)	Mean±SEM			
		0 min (s)	30 min (s)	60 min (s)	90 min (s)
Normal saline	10 mL/kg	6±1.3	9.40±2.69	12.8±2.17	16±3.04
I. hochstetteri	150	9.6±1.36	12.4±1.36	17.8±2.95*	10±0.77
I. hochstetteri	300	13.2±0.86	13.2±0.86*	24.6±1.35*	24.2±1.15*
I. hochstetteri	600	9.6±1.32	12.6±2.27*	26.8±2.35*	26.8±2.86*
Pentazocine	10	8.8±1.28	15.6±2.01*	27±2.27*	26.6±2.01*

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*Significant difference compared to the normal saline control group (one-way ANOVA followed by Dunnett's post hoc test) ($P<0.05$).

2. Xylene-induced ear edema test

Figure 1 shows the effects of *I. hochstetteri* on xylene-induced acute inflammation in rats. Compared to the control group, the inhibition produced by the extract at 300 and 600 mg/kg doses was significant ($P<0.05$), with percentage inhibitions of 40% and 52%, respectively. Dexamethasone treatment (10 mg/kg) inhibited ear edema with an inhibition rate of 72% ($P<0.05$).

Methanol extract of *I. hochstetteri* exhibits both peripheral and central analgesia

1. Acetic acid-induced writhing test

The methanol extract of *I. hochstetteri* (300 and 600 mg/kg) significantly reduced the number of abdominal writhings in mice. The analgesic effect of the extract was dose-dependent (Figure 2).

2. Hot plate test

The results of the hot plate-induced nociception in mice (Table 3) showed that the *I. hochstetteri* whole-plant extract significantly delayed the reaction times to pain for over 90 min of the study. The result at 600 mg/kg was comparable to the standard drug (pentazocine).

Discussion

Pain and inflammation are the major symptoms of most common disease conditions in humans; however, the current conventional drugs are known to have several side effects. *I. hochstetteri* is a well-known plant in Africa, with a long history of utilization in treating diseases. Preliminary phytochemical screening of the methanol extract revealed the presence of glycosides, steroids/terpenes, flavonoids, and saponins, while tannins, alkaloids, and anthraquinones were absent. The presence of terpenes, flavonoids, saponins, alkaloids and tannins was confirmed in previous studies [21]. This disparity could be due to differences in solvents and extraction methods.

In an acute toxicity study, the results suggested that the methanol extract of *I. hochstetteri* has a relatively moderate acute toxicity profile, with an LD_{50} of approximately 2,200 mg/kg [22]. Lower doses up to 1000 mg/kg did not result in mortality, indicating a higher threshold for acute toxicity at these levels. However, doses exceeding 1600 mg/kg begin to show significant toxicity, with complete mortality at 2900 mg/kg and 5000 mg/kg (Table 1).

The formalin-induced paw edema model is a valuable tool in pharmacological research for studying acute inflammatory responses and pain mechanisms. Formalin injection into the hind paw produces a biphasic response: An early neurogenic phase, followed by a later tissue-mediated phase. Upon injection, formalin triggers acute inflammation characterized by increased vascular permeability and plasma extravasation. This process induces the release of inflammatory mediators, such as substance P, prostanoids, serotonin, and histamine, all contributing to edema formation. A neurogenic component involving transient receptor potential ankyrin 1 (TRPA1) receptors is also involved. The rapid initiation of edema is closely linked to early nociception, which relies on primary afferent neurons and axonal reflexes. The down-regulation of the inflammatory response, especially in the later stages dominated by tissue-mediated components, is primarily controlled via supraspinal. This down-regulation involves descending neuronal pathways and potentially a secondary humoral component [23-26].

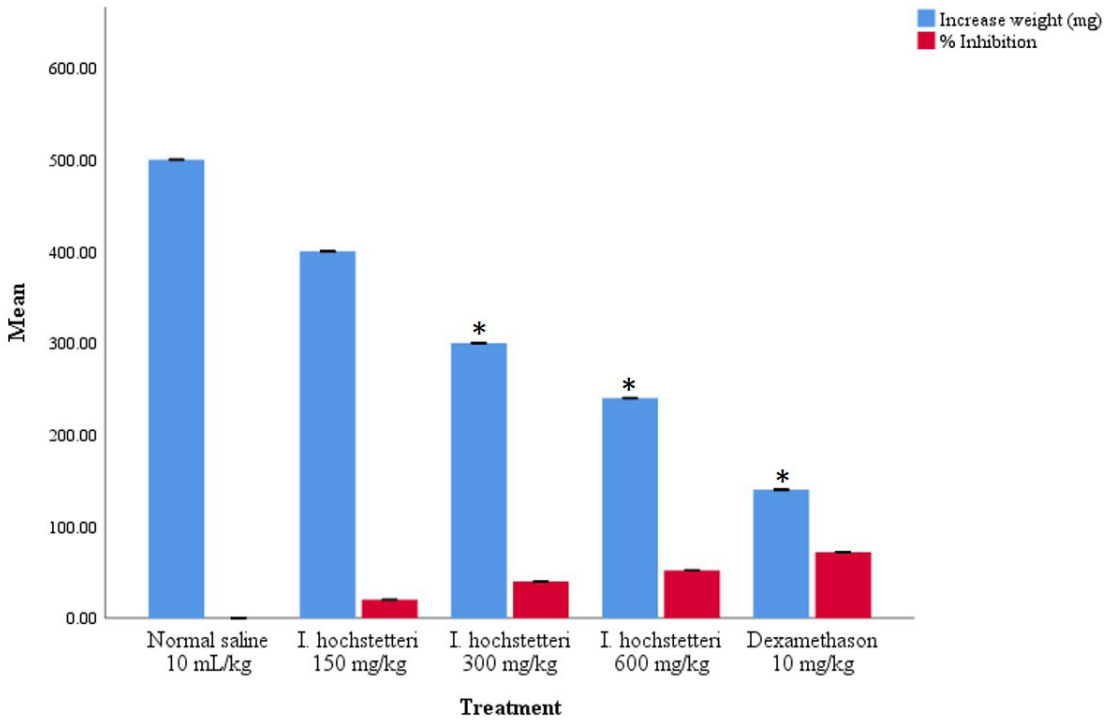

Figure 1. Anti-inflammatory effect of *I. hochstetteri* in xylene-induced ear edema in rats

The results of the anti-inflammatory effects of the methanol extract of *I. hochstetteri* on formalin-induced paw edema in mice showed that at the lower dose of 150 mg/kg, the extract produced a slight decrease in paw size from 4.45±0.11 mm at 0 minutes to 4.21±0.12 mm at 90 minutes. This reduction was significantly only at 90 minutes ($P<0.05$). At the dose of 300 mg/kg, a significant reduction in paw edema was recorded from 30 minutes (4.59±0.04 mm) and continuing through 60 minutes (4.39±0.04 mm) and 90 minutes (4.12±0.02 mm). The highest dose tested (600 mg/kg) significantly reduced the paw edema at all measured time points. Indomethacin (10 mg/kg) as the positive control also significantly reduced paw edema at all time points (Table 2). These results show that *I. hochstetteri* exhibits a dose-dependent anti-inflammatory effect in formalin-induced paw edema in mice. As expected, indomethacin was the most effective in reducing inflammation, showing a substantial decrease in paw size throughout the study. However, the efficacy of *I. hochstetteri* at higher doses approached that of indomethacin, suggesting its potential as a viable natural alternative for inflammation management.

Xylene-induced ear edema is a classical model for acute inflammatory tests used to evaluate the anti-inflammatory potential of drugs and natural products. Xylene exposure causes the upregulation and expression of cyclooxygenase-2 (COX-2), a pro-inflammatory enzyme that catalyzes prostaglandins (PGs) production, contributing to edema formation, and increases myeloperoxidase activity in the ear tissue [26]. Xylene causes a significant increase in the levels of inflammatory mediators, such as nuclear factor kappa B (NF-κB) p65, tumor necrosis factor α (TNF-α), and interleukin-1β (IL-1β) in the ear, as well as induces oxidative stress [26]. Therefore, compounds that inhibit the upregulation of COX-2, NF-κB, pro-inflammatory cytokines, and oxidative stress markers can attenuate the xylene-induced ear edema.

In this study, treatment with 150 mg/kg of the extract resulted in a decrease in ear weight of 400±0.31 mg, corresponding to a 20% inhibition of edema. At 300 and 600 mg/kg doses, the extract significantly reduced ear weight, achieving a 40% and 52% inhibition of inflammation, respectively. This result was statistically significant ($P<0.05$), demonstrating a dose-dependent and strong anti-inflammatory potential of *I. hochstetteri* at higher doses. The progressive decrease in ear edema with increasing extract doses suggests that higher concentrations of the bioactive compounds in *I. hochstetteri* are more effective at mitigating inflammation. As a positive control, dexamethasone, a well-known corticosteroid, substantially decreased ear weight, corresponding to 72% inhibition. This marked reduction ($P<0.05$) underscores the high efficacy of dexamethasone in reducing inflammation, serving as a benchmark for the effica-

cy of *I. hochstetteri* (Figure 1). Comparing the efficacy of *I. hochstetteri* with dexamethasone, it is evident that although the plant extract is less potent than the synthetic corticosteroid, it still offers considerable anti-inflammatory benefits. This is particularly relevant given the side effects associated with long-term corticosteroid use.

The above anti-inflammatory effects observed can be attributed to the phytochemicals found. The recorded phytochemicals (flavonoids, terpenes, saponins, and glycosides) have been reported to possess anti-inflammatory properties via multiple mechanisms, including targeting various inflammatory pathways, modulating inflammatory cytokines, inhibiting inflammatory and antioxidant pathways [27-35]. Furthermore, glycosides inhibited leukocyte function [34], while terpenes and saponins modulated the activity of immune cells [30, 31, 36].

Acetic acid-induced writhing and hot plate tests were used to evaluate the analgesic effects of the extracts. Injected intraperitoneally of acetic acid induces pain by irritating the peritoneal cavity via a peripheral mechanism involving the COX pathway, causing the release of various inflammatory mediators, including PGs (particularly PG E2 [PGE2] and PG I2 [PGI2]), bradykinin, substance P, and histamine [37, 38]. The characteristic writhing or stretching behavior observed in rodents following the intraperitoneal injection of acetic acid indicates the presence of pain, and a reduction in the number of writhes indicates the analgesic effect of the tested substance [38]. In this study, administration of 150 mg/kg of *I. hochstetteri* resulted in a slight reduction in writhes, corresponding to a 16.3% inhibition. Although this dose showed some analgesic effect, it was not statistically significant. The extract at 300 mg/kg and 600 mg/kg, produced a significant reduction ($P<0.05$) in writhing, which corresponded to 45.5% and 76.3% inhibition, respectively. As a positive control, piroxicam significantly reduced the number of writhes, representing an 83.6% inhibition. The results show a clear dose-dependent analgesic effect of the methanol extract of *I. hochstetteri* in the acetic acid-induced writhing test. The significant reduction in the number of abdominal writhes at 300 mg/kg and 600 mg/kg indicates that *I. hochstetteri* has strong peripheral analgesic properties (Figure 2).

The hot plate test is a model of centrally mediated nociception widely used to assess the analgesic effects of compounds by measuring the response to a thermal nociceptive stimulus. The procedure involves placing a rodent on a hot plate maintained at a constant temperature and recording the latency to a nociceptive response, such as paw licking, paw flicking, or jumping. Longer latencies indicate less sensitivity to pain, thus an analgesic effect [39]. The results shown in Table 3 present the data

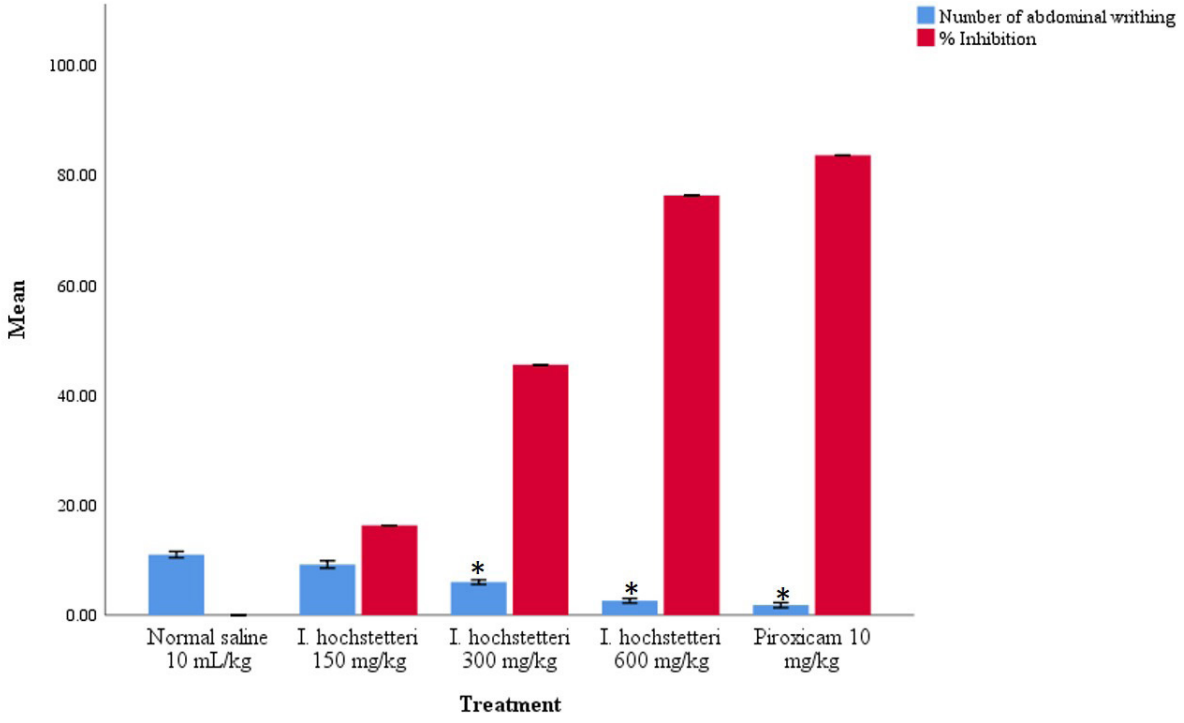

Figure 2. Effect of methanol extract of *I. hochstetteri* on acetic acid-induced writhing test in mice

on the effect of the methanol extract of the whole plant *I. hochstetteri* on pain latency in mice using the hot plate test. Mice treated with normal saline showed increasing pain latency over time, reflecting the natural adaptation to the hot plate stimulus without any analgesic intervention. The 150 mg/kg extract resulted in a significant pain latency only at 60 minutes, suggesting a temporary analgesic effect; however, at 300 and 600 mg/kg, there is a marked increase in pain latency at 30, 60, and 90 minutes. Therefore, the methanol extract of *I. hochstetteri* demonstrated significant analgesic effects in the hot plate test, with higher doses providing sustained pain relief comparable to the standard analgesic, pentazocine.

The observed analgesic effects could be attributed to the phytochemicals present in the extract. Terpenes, glycosides, saponins, and flavonoids have been reported to elicit analgesic effects by inhibiting inflammatory enzymes (such as COX-1, COX-2, and lipoxygenases [LOX]), modulating inflammatory pathways (such as NF-κB and mitogen-activated protein kinase [MAPK]), and depleting endogenous neurotransmitters [32, 40-42]. In addition, saponins interact with arachidonic acid metabolism, glycosides, terpenes, and flavonoids, which inhibit ion channels involved in pain signaling (voltage-gated ion channels, such as sodium, potassium, and calcium channels), and glycosides and flavonoids exert antioxidant properties [32, 40, 41, 43, 44].

Conclusion

The whole-plant methanol extract of *I. hochstetteri* showed significant potential as a natural anti-inflammatory and analgesic agent at the doses investigated. The study's findings highlight the extract's efficacy in reducing inflammation and alleviating pain in animal models, suggesting that it could be a viable alternative to traditional NSAIDs and opioids. The presence of active phytochemicals, such as glycosides, terpenes, flavonoids, and saponins supports its traditional use and indicates its therapeutic potential. Further research should investigate the exact mechanisms of action underlying the anti-inflammatory and analgesic effects of *I. hochstetteri*. The isolation and characterization of the specific bioactive compounds responsible for the observed effects could lead to developing new, targeted anti-inflammatory and analgesic therapies. Also, extended toxicity studies are necessary to assess the long-term safety profile of *I. hochstetteri*, particularly at higher doses.

Ethical Considerations

Compliance with ethical guidelines

The study's ethical protocol was approved by the Faculty of Basic Medical Sciences' Research and Ethics Committee (FBMSREC), Bauchi State University Gadau, Nigeria (Code: BASUG/FBMS/REC/VOL.07/0103).

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Authors' contributions

Conceptualization: Albashir Tahir, Musab Abba Usman, and Suleiman Yunusa; Methodology: Albashir Tahir and Suleiman Yunusa; Software: Albashir Tahir and Nura Abubakar; Validation: Albashir Tahir and Nura Abubakar; Writing the original draft: Albashir Tahir and Khadija Abdullahi Kobi; Review and editing: Albashir Tahir, Nura Abubakar and Suleiman Yunusa; Supervision: Musab Abba Usman and Suleiman Yunusa; Project administration: Albashir Tahir and Khadija Abdullahi Kobi; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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