Evaluation of Acute Toxicity, Anti-Inflammatory and Analgesic Effects of *Ducrosia anethifolia* on Mice

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LINK MEDICAL INTERFACE

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ABSTRACT

Background: Pain and inflammation are complex biological processes often linked with discomfort due to tissue damage. Ducrosia anethifolia, native to Balochistan, Pakistan, is traditionally used for its analgesic and anti-inflammatory properties.

Objective: This study evaluated the acute toxicity, analgesic, and anti-inflammatory effects of the methanolic extract of D. anethifolia seeds in mice.

Methods: D. anethifolia seeds were collected, authenticated, and extracted with methanol. Acute toxicity was assessed by administering oral doses of 100, 500, 1000, 2000, and 3000 mg/kg in mice, monitoring mortality over 24 hours. Analgesic effects were evaluated using acetic acid-induced writhing and formalin tests, with extract doses of 250 mg/kg and 500 mg/kg compared to diclofenac sodium (50 mg/kg). Anti-inflammatory effects were assessed using carrageenan-induced paw edema, measuring paw volume at intervals up to 5 hours post-injection.

Results: The LD50 was 2000 mg/kg. In the acetic acid-induced writhing test, 250 mg/kg and 500 mg/kg doses showed 41.16% ($p<0.05$) and 62.07% ($p<0.01$) inhibition, respectively. The formalin test showed reductions in licking/biting behaviors at 250 mg/kg (39.66 ± 0.60, $p<0.05$) and 500 mg/kg (30.16 ± 1.24, $p<0.05$). The 500 mg/kg dose reduced paw edema by 48.40% ($p<0.05$).

Conclusion: The methanolic extract of D. anethifolia seeds exhibited significant analgesic and anti-inflammatory effects, validating its traditional use and potential for developing safer therapeutic agents.

INTRODUCTION

Pain is a multifaceted experience that encompasses sensory, emotional, and cognitive dimensions, arising from tissue damage and acting as a critical warning signal of underlying problems within the body (1). Pain can be transient, subsiding as the harmful stimulus is removed or the tissue damage heals, yet millions of individuals endure chronic pain, necessitating frequent medical consultations (2). The complex interplay of social, physical, and psychological factors contributing to pain, alongside its progression to disability, remains insufficiently understood, with specific biopsychosocial elements potentially playing a pivotal role (3). Inflammation, a highly conserved physiological response among living organisms, is triggered by tissue injury and serves as a protective mechanism. However, when unchecked, it can escalate to destructive levels, causing organ failure and death (4). The activation of immune, neurological, and endocrine mediators during inflammation can exacerbate the condition, leading to chronic inflammation and sepsis, a severe and often fatal outcome. In the United States alone, approximately 750,000 cases of sepsis are reported annually (5).

The American Pain Society reports that 50 million individuals across various age groups suffer from pain, with an estimated $25 million required for its management (6). Currently, pain management predominantly relies on narcotic opioids or non-narcotic salicylates and corticosteroids, such as hydrocortisone, all of which carry significant adverse reactions and toxic effects. Both short- and long-term use of opioids can result in complications including reduced gastrointestinal activity, nausea, vomiting, pruritus, and dependence (7). Consequently, there is a growing reliance on medicinal plants, with the World Health Organization (WHO) noting that three-quarters of the global population, including 25% of the United States' population, depend on plant-derived medications for various ailments (8). Eighty percent of people in developing countries utilize herbal products for medical needs, with more than 30% of total plant species employed for medicinal purposes. The current market value of medicinal plants stands at an impressive $20 million (9).

Pakistan, with its diverse climate zones and unique biodiversity, is home to around 6000 medicinal plant species, of which 600 to 700 are used for medicinal purposes (10). Balochistan, the largest province in Pakistan, covers 44% of the country's total area and hosts a significant number of these medicinal plants due to its varied climatic conditions. *Ducrosia anethifolia*, a member of the Apiaceae family, is one of the major medicinal plants found in the Makran region of Balochistan. Locally known as Gowatik, KhorKundai, and Gwartag, this plant is widely used for its analgesic and anti-inflammatory properties, particularly for treating stomach pain (11).

Previous studies on Ducrosia species, including *D. anethifolia* and D. ismaelis, have identified various bioactive compounds such as alkaloids, terpenoids, flavonoids, and phytosterols. These compounds, particularly flavonoids and alkaloids, are believed to contribute significantly to the plant's analgesic properties (12). Given the widespread use of *D. anethifolia* in traditional medicine and the need for scientifically validated alternatives to conventional pain management therapies, this study aims to evaluate the acute toxicity, analgesic, and anti-inflammatory effects of the methanolic extract of *D. anethifolia* seeds on mice.

The acute toxicity assessment involved administering varying doses of the seed extract to determine its safety profile, while the analgesic effects were evaluated using acetic acid-induced writhing and formalin-induced pain tests. The anti-inflammatory effects were assessed using the carrageenan-induced paw edema model. This comprehensive approach provides a detailed understanding of the pharmacological potential of *D. anethifolia*, supporting its traditional use and highlighting its potential as a safer alternative to conventional pain and inflammation treatments (13). The findings of this study are expected to contribute to the growing body of knowledge on medicinal plants and their application in modern pharmacology, ultimately aiding in the development of new therapeutic agents with fewer side effects.

MATERIAL AND METHODS

The seeds of *Ducrosia anethifolia* were sourced from the local market in the Panjgur district of Balochistan, Pakistan. Specimens were collected from Sabzap, Panjgur, and authenticated by Professor Shafi Mahmad from the Department of Pharmacognosy, University of Balochistan, with the specimen number MD-185/2019. The seeds were ground and soaked in methanol for 15 days to prepare the methanolic extract, which was subsequently concentrated using a rotary evaporator.

Experimental animals, including male and female mice weighing between 25 to 30 grams, were procured from the Dow University of Health Sciences, Karachi. These mice were housed under standard laboratory conditions with a temperature of 30°C, a 12-hour light/dark cycle, and free access to food and water. Animals were acclimatized for seven days before the commencement of the experiments. All procedures involving animals were conducted following the guidelines of the Helsinki Declaration and were approved by the Institutional Animal Care and Use Committee (IACUC). The acute toxicity of the *D. anethifolia* methanolic seed extract was assessed using the method described by Lorke (21). Six groups of six mice each were administered varying doses of the extract (100, 500, 1000, 2000, and 3000 mg/kg) orally, while the control group received 0.5 ml of normal saline. The animals were observed for 24 hours to record mortality and calculate the median lethal dose (LD50) (11).

To evaluate the analgesic activity, the acetic acid-induced writhing test was performed as per the method of Koster (22). Mice were divided into four groups, with the control group receiving 0.5 ml of normal saline, and the other groups receiving 250 mg/kg or 500 mg/kg of *D. anethifolia* extract, or 50 mg/kg of the standard drug diclofenac sodium. Thirty minutes post-administration, 0.6% glacial acetic acid (10 ml/kg) was injected intraperitoneally, and the number of abdominal writhes was recorded for 30 minutes. The percentage inhibition of writhes was calculated (23, 24).

The formalin test, used to further assess analgesic activity, involved the injection of 20 µl of 1% formaldehyde diluted in normal saline into the dorsal hind paw of the mice (26). Groups were treated similarly to the acetic acid test, with the number of licking and biting behaviors recorded in two phases: the initial acute phase (0-5 minutes) and the chronic phase (15-30 minutes) post-formalin injection (27, 28).

Anti-inflammatory activity was evaluated using the carrageenan-induced paw edema method described by Winter (29). Mice were divided into four groups, treated with either normal saline, 250 mg/kg or 500 mg/kg of *D. anethifolia* extract, or 10 mg/kg of diclofenac sodium. One hour after treatment, 0.1 ml of 1% carrageenan was injected into the subplantar region of the left hind paw. Paw volume was measured at 0, 1, 2, 3, 4, and 5 hours post-carrageenan injection using a digital vernier caliper. The percentage inhibition of paw edema was calculated (30). Data collected from these experiments were analyzed using SPSS version 25.0. Results were expressed as mean ± standard error of the mean (SEM). Statistical significance was determined using one-way ANOVA followed by post-hoc tests, with p-values less than 0.05 considered significant.

RESULTS

The study evaluated the acute toxicity, analgesic, and anti-inflammatory effects of *Ducrosia anethifolia* methanolic seed extract on mice. The detailed results are presented below. The acute toxicity study revealed that the crude extract of *D. anethifolia* seed was safe at doses up to 1000 mg/kg. Mortality was observed at 2000 mg/kg (50%) and 3000 mg/k(83%), establishing the LD50 at 2000 mg/kg

Table 1 Acetic Acid-Induced Writhing Test

Treatment	Oral Dose	Number of Mice	Mortality (%)
Control (0.5 ml normal saline)	-	6	0%
Crude extract of *D. anethifolia*	200 mg/kg	6	0%
	500 mg/kg	6	0%
	1000 mg/kg	6	0%
	2000 mg/kg	6	50%
	3000 mg/kg	6	83%

The methanolic seed extract of *D. anethifolia* demonstrated significant analgesic effects. At a dose of 250 mg/kg, the number of writhes reduced significantly ($p<0.05$) with a percentage inhibition of 41.16%. A higher dose of 500 mg/kg showed a more significant effect ($p<0.01$) with a percentage inhibition of 62.07%. The standard drug, diclofenac sodium, showed a percentage inhibition of 67.24%.

Table 2 Formalin Test

Treatment	Dose	Mean No. of Writhes ± SEM	Percentage Inhibition (%)
Control (0.5 ml normal saline)	-	77.33 ± 1.16	0
Crude extract of *D. anethifolia*	250 mg/kg	45.5 ± 2.76*	41.16
	500 mg/kg	29.33 ± 0.6**	62.07
Diclofenac sodium	50 mg/kg	25.33 ± 1.08**	67.24

In the formalin test, both doses of *D. anethifolia* methanolic seed extract showed significant analgesic effects in both the acute and chronic phases. The number of licking and biting behaviors significantly reduced in treated groups compared to the control, with higher efficacy observed at the 500 mg/kg dose.

Table 3 Anti-inflammatory Activity

Treatment	Dose	First Phase (0-5 min)	Second Phase (15-30 min)
		Number of Licking & Biting	Time Taken (sec)
Control (0.5 ml normal saline)	-	43.83 ± 3.0	51.66 ± 2.70
Crude extract of *D. anethifolia*	250 mg/kg	29.83 ± 0.94	48.66 ± 3.68
	500 mg/kg	25.66 ± 0.6	32.33 ± 1.62
Diclofenac sodium	50 mg/kg	20.5 ± 0.86**	18 ± 0.57**

The anti-inflammatory effects were assessed using the carrageenan-induced paw oedema method. The extract at 500 mg/kg showed a significant reduction in paw oedema ($p<0.05$) with a percentage inhibition of 48.40%, while the standard drug diclofenac sodium demonstrated a percentage inhibition of 56.63%.

Table 4 Paw Volume at Different Hours

Treatment	Dose	Paw Volume (Mean ± SEM)	Inhibition (%)
		0 hour	1 hour
Control (0.5 ml normal saline)	-	2.34 ± 0.01	3.71 ± 0.12
Crude extract of *D. anethifolia*	250 mg/kg	2.38 ± 0.01	3.48 ± 0.11
	500 mg/kg	2.40 ± 0.01	3.43 ± 0.2
Diclofenac sodium	10 mg/kg	2.29 ± 0.01	3.22 ± 0.08

DISCUSSION

The study's findings revealed that the methanolic seed extract of *Ducrosia anethifolia* demonstrated significant analgesic and anti-inflammatory properties, supporting its traditional use in pain management and inflammation control. The acute toxicity assessment indicated that the extract was safe at doses up to 1000 mg/kg, with an LD50 of 2000 mg/kg, establishing a foundational understanding of the extract's safety profile (21).

The analgesic effects observed in the acetic acid-induced writhing test and formalin test were consistent with previous studies on other medicinal plants within the Apiaceae family. The reduction in the number of writhes and the licking and biting behaviors in both phases of the formalin test highlighted the extract's potential in managing both acute and chronic pain. These findings align with studies on Ducrosia ismaelis and Cuminum cyminum, which have shown significant analgesic effects attributed to their flavonoid and alkaloid contents (12, 32). The involvement of these compounds in modulating pain pathways through the inhibition of pro-inflammatory mediators such as bradykinin, serotonin, histamine, and prostaglandins is well-documented (34).

In the anti-inflammatory assessment using the carrageenan-induced paw edema model, the extract at 500 mg/kg significantly inhibited paw edema, further corroborating its anti-inflammatory potential. The efficacy observed was comparable to that of diclofenac sodium, a standard anti-inflammatory drug. This result is consistent with previous research on *Ducrosia anethifolia* essential oils, which have shown similar anti-inflammatory effects (31). The anti-inflammatory action can be attributed to the presence of bioactive compounds that modulate inflammatory responses, reducing the release of pro-inflammatory cytokines and mediators (36).

The acute toxicity study was limited to a single administration route and did not explore chronic toxicity or long-term effects. Additionally, the study did not identify the specific bioactive compounds responsible for the observed effects, nor did it explore their mechanisms of action in detail. Future research should focus on isolating and

characterizing these compounds to better understand their pharmacodynamics and pharmacokinetics. Furthermore, extending the toxicity studies to include chronic and sub-chronic assessments would provide a more comprehensive safety profile (21, 36).

The study's strength lies in its comprehensive approach, utilizing well-established models to evaluate both analgesic and anti-inflammatory activities. The consistency of the results with previous findings on related species enhances the reliability of the conclusions drawn. However, variability in the source and quality of plant materials, potential differences in extraction methods, and the lack of standardization in dosing regimens can influence the reproducibility of results across different studies.

The methanolic seed extract of *Ducrosia anethifolia* exhibited significant analgesic and anti-inflammatory effects, validating its traditional use in pain and inflammation management. These findings suggest that *D. anethifolia* could serve as a potential source for developing new therapeutic agents with fewer side effects compared to conventional drugs. However, further studies are necessary to isolate the active compounds, elucidate their mechanisms of action, and assess their long-term safety and efficacy in larger animal models and clinical trials. The integration of advanced analytical techniques and interdisciplinary research approaches will be crucial in advancing the pharmacological understanding of this medicinal plant (12, 21, 31, 36).

CONCLUSION

The study concluded that the methanolic seed extract of *Ducrosia anethifolia* exhibits significant analgesic and anti-inflammatory properties, supporting its traditional use in managing pain and inflammation. These findings indicate the potential for *D. anethifolia* to serve as a source for developing new therapeutic agents that may offer fewer side effects compared to conventional medications. The positive results from this study suggest promising implications for human healthcare, particularly in providing alternative pain relief and anti-inflammatory treatments. Future research should focus on isolating active compounds, understanding their mechanisms, and conducting clinical trials to fully realize the potential benefits of *D. anethifolia* in medical practice.

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