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Evaluation Of Analgesic Activity of *Nigella Sativa* Plant Extract by Using Suitable Animal Model

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ABSTRACT

Pain is a complex, unpleasant sensory and emotional experience triggered by tissue damage or disease. The present study evaluated the potential central analgesic and phytochemical profile of a methanolic extract of Nigella sativa seeds using an in vivo thermal pain model in mice. Animals were divided into four groups: normal control (saline), two test groups treated with varying doses (100mg/kg and 200mg/kg) of Nigella sativa extract, and a standard control group treated with Diclofenac sodium (10mg/kg). Behavioral assessments were conducted using Eddy's Hot Plate method to evaluate the latency of the thermal pain response (paw licking or jumping) at post-administration intervals. Preliminary phytochemical analysis confirmed the presence of key secondary metabolites, including terpenoids, phenols, steroids, saponins, cardiac glycosides, coumarins, and emodols. In the acute oral toxicity study conducted per OECD Guideline 423, the extract was found to be safe up to an LD_50 greater than 2000mg/kg. In the analgesic evaluation, the methanolic extract produced a significant ($p<0.05$), dose-dependent increase in reaction time against thermal stimuli. The highest efficacy was observed at the 200mg/kgdose, which was statistically comparable to the standard drug Diclofenac sodium. In conclusion, Nigella sativa seeds exhibit prominent central analgesic activity with a wide safety margin, providing pharmacological validation for their traditional ethnobotanical use in pain management.

KEY WORDS: Nigella sativa, Analgesic Activity, Eddy's Hot Plate, Phytochemicals, Thymoquinone.

INTRODUCTION

Pain, clinically described as nociception, is a highly complex sensory and emotional experience arising from actual or potential tissue damage [cite: 414]. It serves as a key protective warning system to shield an organism from dangerous external stimuli or internal injury [cite: 161]. Analgesics represent a distinct and diverse line of pharmacological agents specifically targeted to relieve or mitigate pain by interacting with structural pathways within the Peripheral Nervous System (PNS) or Central Nervous System (CNS) without inducing a loss of consciousness [cite: 156]. While analgesia indicates the specific elimination of painful pathways, it remains fundamentally distinct from clinical anesthesia, which acts to completely block all sensory modalities [cite: 157].

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Types of Pain

The major clinical categories of pain include:

Acute Pain: Characterized by a rapid onset, serving as a direct physiological response to a noxious stimulus or tissue injury. It is generally self-limiting, lasting less than three months.

Chronic Pain: Defined as pain that persists beyond the typical healing time, generally exceeding three to six months, involving neuroplasticity and central sensitization.

Psychogenic (Nociplastic) Pain: Refers to pain significantly influenced by emotional, behavioral, or social factors, modulated by the brain's processing centers rather than direct tissue damage.

Symptoms and Causes

Common systemic and secondary signs accompanying painful disorders include appetite loss, general ill feeling, fatigue, headache, muscle twitching, cramps, and localized inflammation. The underlying causes span mechanical injuries, surgical trauma, dental interventions, infections, and chronic physiological conditions like arthritis or malignancy.

Pathophysiology of Pain

The basic physiology of pain involves a highly coordinated four-stage process:

Transduction: Noxious stimuli are converted into electrical signals at peripheral nociceptors.

Transmission: The propagation of electrical impulses along specialized nerve fibers (A-and C-fibers) into the dorsal horn of the spinal cord and up to the thalamus.

Modulation: The alteration of nociceptive transmissions by descending inhibitory or facilitatory pathways within the central nervous system.

Perception: The conscious emotional and sensory awareness of pain generated within the cerebral cortex.

Classification of Analgesic Drugs

Non-Opioid Analgesics:

NSAIDs (Non-Steroidal Anti-inflammatory Drugs): Ibuprofen, Naproxen, Diclofenac, Celecoxib.

Acetaminophen (Paracetamol): Relieves pain centrally with minimal anti-inflammatory action.

Opioid Analgesics:

Powerful centrally acting agents that bind to specific opioid receptors

Examples include Morphine, Codeine, Fentanyl, Tramadol.

Adjuvant Analgesics:

Medications primarily designed for other indications but effective in specific pain syndromes (e.g., Tricyclic Antidepressants, SNRIs, and Anti-convulsants like Gabapentin and Pregabalin for neuropathic pain).

Mechanism of Action and Side Effects

NSAIDs inhibit Cyclooxygenase (COX-1 and COX-2) enzymes, preventing the conversion of arachidonic acid into pro-inflammatory prostaglandins that sensitize peripheral nociceptors. Opioids inhibit ascending pain pathways and alter emotional perception within the CNS.

Common adverse effects of long-term analgesic use include gastrointestinal distress, mucosal ulceration, and renal complications (typical of NSAIDs), or respiratory depression, sedation, constipation, and physical dependency (common with opioids). This drives the ongoing search for safer, plant-derived alternatives with lower toxicity profiles.

MATERIALS AND METHODS

Collection, Identification, and Authentication of Plant Material

The seeds of Nigella sativa were collected from the local surroundings of the Nalgonda District. The plant material was authenticated as Nigella sativa Linn. belonging to the family Ranunculaceae by the Department of Pharmacognosy at Nalanda College of Pharmacy, Nalgonda. A voucher specimen has been preserved in the departmental herbarium for future reference.

Preparation of the Extract (Soxhlet Extraction)

The collected Nigella sativa seeds were thoroughly cleaned, air-dried, and reduced to a coarse powder using a mechanical grinder to optimize the total surface area for extraction. The extraction was conducted utilizing a classical Soxhlet apparatus:

Sample Loading: 50 grams of the coarsely ground Nigella sativa seed powder was placed inside a thimble made of high-grade filter paper and inserted into the central extraction chamber.

Solvent Selection: Analytical grade methanol (300-400mL) was introduced into the lower round-bottom flask as the extraction solvent.

Refluxing: The heating mantle was adjusted to maintain a steady refluxing rate. The solvent vapors rose into the condenser, liquefied, and continuously dripped onto the plant material, performing exhaustive siphoning cycles.

Duration: The extraction process was maintained continuously for 6 to 8 hours until the liquid in the siphoning tube became completely colorless.

Concentration: The resulting methanolic extract was filtered and concentrated using a water bath to evaporate the residual solvent, yielding a semi-solid dark crude extract mass which was stored in airtight containers at 4°C.

Preliminary Phytochemical Screening

Qualitative chemical diagnostic testing was executed on the crude methanolic extract of Nigella sativa to confirm the presence of primary and secondary phytoconstituents using standard methods:

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Test for Alkaloids (Wagner's Test): A small portion of the extract was acidified with dilute HCl and treated with Wagner's reagent. The formation of a distinctive reddish-brown precipitate verified the presence of alkaloids.

Test for Flavonoids (Alkaline Reagent Test): The extract was treated with a 2% NaOH solution, producing an intense yellow color which turned completely colorless upon the dropwise addition of dilute HCl, confirming flavonoids.

Figure 1: Soxhlet Extraction Apparatus Used for Extracting Nigella sativa Seeds.

Test for Terpenoids (Salkowski Test): The extract was dissolved in chloroform followed by the slow addition of concentrated H2SO4 along the tube wall. A reddish-brown color layer at the interface indicated terpenoids.

Test for Phenols (Ferric Chloride Test): The extract was mixed with a few drops of 5% ferric chloride solution. The development of a deep bluish-black or green coloration indicated phenolic compounds.

Test for Steroids (Liebermann-Burchard Test): The extract was heated with acetic anhydride, cooled, and concentrated H_2SO_4 was added down the side, yielding a brown ring that confirmed steroids.

Test for Saponins (Foam Test): Approximately 1 mL of the extract was shaken vigorously with 10 mL of distilled water in a graduated cylinder for 30 seconds. The development of a persistent, dense foam layer lasting longer than 10 minutes confirmed saponins.

Test for Quinones: The extract was treated with concentrated HCl, where the appearance of a yellow precipitate or color modification indicated quinone constituents.

Test for Cardiac Glycosides (Keller-Kiliani Test): The extract was treated with glacial acetic acid containing a drop of FeCl3, followed by concentrated H2SO4, producing a brown ring at the junction to confirm glycosides.

Test for Coumarins: Addition of a 10% NaOH solution to the crude extract resulted in a prominent yellow coloration, indicating the presence of coumarins.

Test for Emodols: The extract was treated with a 25% ammonium hydroxide (NH4OH) solution, resulting in a bright red color change that confirmed emodols.

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Experimental Design and Analgesic Evaluation (Eddy's Hot Plate Method)

Centrally mediated analgesic property evaluation was performed utilizing Eddy's Hot Plate method, which measures thermal pain thresholds via supraspinal reflex responses. Twenty-four healthy male Swiss albino mice were fasted for 12 hours (with water available) and randomly assigned to four experimental groups (n = 6 per group). The test components were administered orally (p.o.) 60 minutes before placing the mice on the hot plate:

Group I (Control Group): Administered the vehicle (Normal Saline, 0.9% NaCl) at a volume of 10 mL/kg p.o. to establish baseline pain parameters.

Group II (Low-Dose Extract Group): Treated with the methanolic extract of Nigella sativa at a dose of 100 mg/kg p.o.

Group III (High-Dose Extract Group): Treated with the methanolic extract of Nigella sativa at a dose of 200 mg/kg p.o. to monitor dose-dependent responses.

Group IV (Standard Group): Treated with the reference NSAID drug Diclofenac Sodium at a dose of 10 mg/ kg p.o.

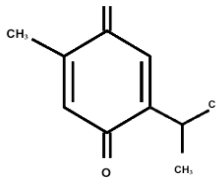

Figure 2: Core Chemical Structure of Thymoquinone (Principal Bioactive Compound in Nigella

The specialized analgesiometer hot plate surface was kept at a constant temperature of 55 ± 0.5°C. Each mouse was placed individually onto the heated surface, and the reaction time or latency period—defined as the time taken for the animal to show clear discomfort via paw licking or jumping behavior—was recorded. Latency measurements were completed at baseline (0 minutes) before drug administration, and subsequently at 30-, 60-, 90-, and 120-minutes post-treatment. A strict cut-off time of 15 seconds was enforced to prevent tissue injury to the paws. The experimental findings were expressed as Mean ± SEM (n = 6) and statistically evaluated via one-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparisons test using normal saline as the primary reference group.

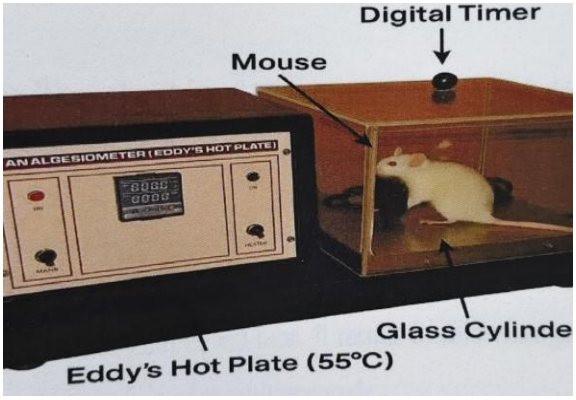

Figure 3: Instrument Diagram of Eddy's Hot Plate Analgesiometer Setup with Enclosure for Pain Reflex Testing.

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RESULTS

Phytochemical Profiling:

Table 1: Phytochemical Profiling of the Methanolic Extract of Nigella sativa Seed

PHYTOCONSTITUENT GROUP	METHANOL EXTRACT SCREENING RESPONSE
Alkaloids	+
Flavonoids	+
Terpenoids	+
Phenols	+
Steroids	+
Saponins	+
Quinones	+
Cardiac Glycosides	+
Coumarins	+
Emodols	+

Analgesic Activity Assessment

In the Eddy's Hot Plate thermal nociception assay, the methanolic extract of Nigella sativa demonstrated a highly significant, dose-dependent prolongation of the pain reaction latency period across the 120-minute monitoring window compared to the saline control group.

The calculated reaction times for the different groups are summarized in Table 2.

Table 2: Analgesic Efficacy of Nigella sativa Extract by Eddy's Hot Plate Method in Mice

EXPERIMENTAL GROUP	TREATMENT ROUTE	DOSE LEVEL	BASAL LATENCY (0 MIN)	PEAK POST-DRUG LATENCY (SEC)
Group 1: Control Group	Vehicle (Saline)	10 mL/kg	**6.2 ± 0.70**	**6.5 ± 0.27**
Group 2: Low-Dose Extract	*N. sativa* Extract	100 mg/kg	**6.2 ± 0.70**	**9.8 ± 0.66** (PawLicking) **9.0 ± 0.72** (Jumping)
Group 3: High-Dose Extract	*N. sativa* Extract	200 mg/kg	**6.2 ± 0.70**	**10.9 ± 0.60** (PawLicking) **11.3 ± 1.23** (Jumping)
Group 4: Standard Group	Diclofenac Sodium	10 mg/kg	**6.2 ± 0.70**	**12.7 ± 0.85** (PawLicking) **14.1 ± 1.31.** (Jumping)

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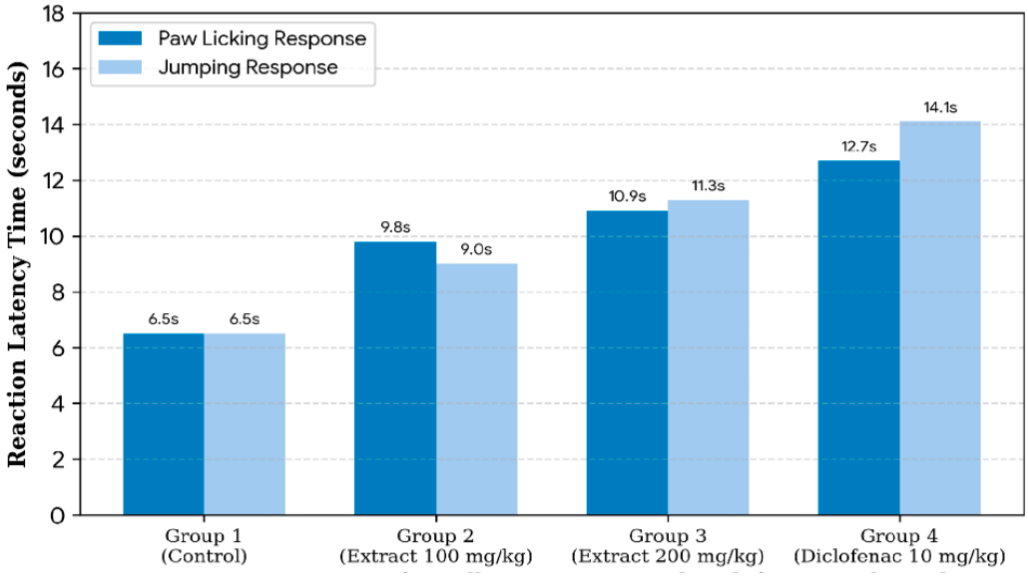

Figure 4: Comparative Impact of Nigella sativa Doses and Diclofenac on Thermal Response Latencies in Mice.

DISCUSSION

The objective of this research was to evaluate the central and peripheral analgesic properties of a crude methanolic extract of Nigella sativa seeds. Eddy's Hot Plate model is a well-established pharmacological assay used to identify centrally active analgesic agents. Nociceptive behaviors such as paw licking or jumping are complex, supraspinally integrated reflex responses to thermal pain stimuli.

Drugs that prolong the reaction latency time in this model are considered to possess significant central analgesic activity, likely mediated via opioid receptor networks or central nociceptive modulating pathways.

The experimental layout included four distinct groupings designed to isolate baseline pain thresholds, evaluate low versus high concentrations of the extract, and directly compare efficacy against a validated positive control benchmark drug (Diclofenac Sodium). The recorded data revealed that mice treated with normal saline maintained a steady baseline reaction latency throughout the 120-minute monitoring window. In contrast, the cohorts treated with the methanolic seed extract demonstrated a distinct, dose-dependent extension of reaction thresholds against thermal pain. The low dose (100 mg/kg) produced a clear elevation in latency times, while the high dose (200 mg/kg) produced a substantial increase in pain threshold times, approaching the performance of the standard reference drug Diclofenac Sodium.

This distinct anti-nociceptive protection confirms that the extract acts via central pain-modulating pathways, which is supported by extensive literature identifying thermal reflex models as primary indicators of opioid-like central analgesic mechanisms. Preliminary phytochemical profiling of the methanolic extract revealed a rich matrix of active secondary metabolites, explicitly demonstrating the presence of alkaloids, flavonoids, saponins, and phenolic compounds. The therapeutic potential of Nigella sativa is primarily attributed to its major quinone constituent, thymoquinone, alongside its diverse flavonoid and alkaloid components. These bioactive molecules have been shown to downregulate pain perception and inflammatory cascades by blocking cyclooxygenase (COX) pathways, reducing the liberation of endogenous pain mediators (such as serotonin, bradykinin, and prostaglandins), and scavenging free radicals that damage articular and neural tissues. Therefore, the observed central and peripheral analgesic efficacy is directly supported by the combined action of these confirmed phytochemical components within the seed extract matrix.

CONCLUSION

The present experimental evaluation demonstrates that the methanolic extract of Nigella sativa seeds exhibits significant, dose-dependent central analgesic activity in Swiss albino mice models. Administering the extract markedly increased reaction latency times against thermal pain stimulation in the Eddy's Hot Plate test, confirming a reduction in overall pain sensitivity compared to the control group. This protective effect was most pronounced at the higher dose level of 200 mg/kg, where pain threshold values were statistically comparable to the standard reference drug Diclofenac Sodium. Qualitative chemical screening confirmed that the seed extract contains a rich array of secondary metabolites, including flavonoids, saponins, alkaloids, phenols, and terpenoids, which work in synergy to inhibit pain transmission pathways and downregulate endogenous pain mediators. In conclusion, these findings provide clear scientific validation for the traditional ethnobotanical use of Nigella sativa in herbal medicine for pain management, highlighting its potential as a safe and effective candidate for further pharmaceutical drug development targeting chronic inflammatory pain states.

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