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ASSESSMENT OF WOUND HEALING ACTIVITY OF *NIGELLA SATIVA* ROOT EXTRACTS USING EXPERIMENTAL RAT MODELS

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ABSTRACT

Nigella sativa L. is a well-known medicinal plant possessing antioxidant, anti-inflammatory, and antimicrobial properties. While extensive research has been conducted on its seeds and seed oil, the pharmacological potential of its roots remains largely unexplored. The present study aimed to scientifically evaluate the wound healing activity of *Nigella sativa* root extracts using experimental animal models. Roots of *Nigella sativa* were successively extracted with petroleum ether, chloroform, ethyl acetate, and ethanol using Soxhlet extraction. The extracts were subjected to preliminary phytochemical screening and acute oral toxicity studies following OECD Guideline 423. Wound healing activity was evaluated in Wistar rats using excision and incision wound models. Parameters including percentage wound contraction, epithelialization period, wound breaking strength, and histopathological changes were assessed and compared with standard and control groups. Phytochemical screening revealed the presence of major bioactive constituents, including flavonoids, phenolic compounds, tannins, alkaloids, saponins, and terpenoids. The extracts were found to be safe at the tested dose levels. Treatment with *Nigella sativa* root extracts significantly enhanced wound contraction, shortened the epithelialization period, improved wound breaking strength, and promoted collagen deposition, fibroblast proliferation, angiogenesis, and re-epithelialization compared with the control group. The findings demonstrate that *Nigella sativa* root extracts possess significant wound healing activity, which may be attributed to their phytochemical constituents with antioxidant and anti-inflammatory properties. These results provide scientific evidence supporting the therapeutic potential of

Nigella sativa roots as a promising natural agent for wound management and justify further studies to isolate and characterize the bioactive compounds responsible for these effects.

KEYWORDS: *Nigella sativa*; Root extract; Wound healing; Animal wound model; Phytochemical screening.

INTRODUCTION

Wound healing is a dynamic and highly coordinated biological process involving hemostasis, inflammation, proliferation, and tissue remodeling. Successful healing requires synchronized cellular and molecular events, including fibroblast proliferation, collagen synthesis, angiogenesis, and re-epithelialization. However, delayed wound healing caused by persistent inflammation, microbial infection, oxidative stress, diabetes, and other pathological conditions remains a significant clinical challenge worldwide.[1] Although conventional wound management therapies are effective, their prolonged use may be associated with adverse effects, high treatment costs, and the emergence of antimicrobial resistance. Consequently, there is increasing interest in identifying safe and effective plant-derived therapeutic agents with wound healing potential. *Nigella sativa* L. (Ranunculaceae), commonly known as black cumin or black seed, is an important medicinal plant widely used in traditional systems of medicine.[2] The plant is rich in biologically active phytoconstituents, including thymoquinone, flavonoids, phenolic compounds, alkaloids, saponins, and terpenoids, which possess antioxidant, anti-inflammatory, antimicrobial, and immunomodulatory activities. Numerous experimental studies have demonstrated the wound healing efficacy of *Nigella sativa* seeds, seed oil, and isolated thymoquinone through enhancement of collagen synthesis, fibroblast proliferation, angiogenesis, and epithelial regeneration. Despite these findings, scientific investigations on the pharmacological potential of *Nigella sativa* roots remain scarce.[3-4]

Different parts of medicinal plants often differ considerably in their phytochemical composition and biological activities. The roots of *Nigella sativa* may contain unique secondary metabolites that could contribute to tissue regeneration and wound repair, yet their therapeutic potential has received little scientific attention. Therefore, systematic evaluation of root extracts is essential to establish their efficacy and provide scientific evidence supporting their traditional medicinal use.[5] The present study was undertaken to investigate the wound healing activity of successive solvent extracts of *Nigella sativa* roots using standard excision and incision wound models in Wistar rats. Preliminary phytochemical

screening, acute toxicity assessment, and histopathological evaluation were performed to correlate the phytochemical constituents with wound healing efficacy. The findings of this study may contribute to the development of novel plant-based therapeutic agents for effective wound management and provide a scientific basis for the medicinal application of *Nigella sativa* roots.

Experimental Work

Fresh roots of *Nigella sativa* were collected, authenticated, shade-dried, powdered, and subjected to successive Soxhlet extraction using petroleum ether, chloroform, ethyl acetate, and ethanol.[6] Preliminary phytochemical screening was performed using standard qualitative methods to identify the presence of major secondary metabolites, including alkaloids, flavonoids, phenolic compounds, tannins, glycosides, saponins, steroids, terpenoids, carbohydrates, and proteins. Acute oral toxicity studies were carried out in Wistar rats according to OECD Guideline 423 to establish the safety profile of the extracts and determine suitable dose levels for pharmacological evaluation.[7]

The wound healing activity was assessed in healthy Wistar albino rats using standard excision and incision wound models. Animals were divided into control, standard, and extract-treated groups.[8] Povidone-iodine ointment (5% w/w) was used as the standard treatment, while the test groups received formulations containing low and high doses of *Nigella sativa* root extract. The healing response was evaluated by measuring percentage wound contraction, epithelialization period, and wound breaking strength. Histopathological examination of healed skin tissues was performed to assess epithelial regeneration, collagen deposition, fibroblast proliferation, angiogenesis, and inflammatory cell infiltration.[9-10] The experimental data were statistically analyzed, and the efficacy of the extract-treated groups was compared with the control and standard treatment groups.

RESULTS

Extractive Yield and Phytochemical Screening: Successive Soxhlet extraction of *Nigella sativa* roots using solvents of increasing polarity produced petroleum ether, chloroform, ethyl acetate, and ethanolic extracts with varying extractive yields. Among the tested solvents, the ethanolic extract exhibited the highest percentage yield (8.92 ± 0.26% w/w), followed by ethyl acetate (5.24 ± 0.19% w/w), chloroform (3.76 ± 0.21% w/w), and petroleum ether (2.48 ± 0.15% w/w). The higher yield obtained with ethanol suggests that the roots contain a

greater proportion of polar phytoconstituents, making ethanol the most suitable extraction solvent for subsequent pharmacological investigations.

Table 1: Percentage yield of successive extracts of *Nigella sativa* roots.

Extract	Colour	Consistency	Percentage yield (% w/w)
Petroleum ether	Brownish yellow	Sticky mass	2.48 ± 0.15
Chloroform	Dark brown	Semisolid	3.76 ± 0.21
Ethyl acetate	Brown	Semisolid	5.24 ± 0.19
Ethanolic	Dark brown	Semisolid	8.92 ± 0.26

Qualitative phytochemical screening demonstrated the presence of several biologically active secondary metabolites in different solvent extracts. The ethanolic extract possessed the richest phytochemical profile, showing abundant flavonoids and phenolic compounds together with alkaloids, tannins, glycosides, saponins, terpenoids, carbohydrates, proteins, and steroids. Petroleum ether extract contained only a limited number of constituents, whereas ethyl acetate and chloroform extracts showed moderate phytochemical diversity. These observations justified the selection of the ethanolic extract for pharmacological evaluation.

Table 2: Preliminary phytochemical screening of *Nigella sativa* root extracts.

Phytoconstituent	Petroleum Ether	Chloroform	Ethyl Acetate	Ethanol
Alkaloids	–	+	+	++
Flavonoids	–	+	++	+++
Phenolic compounds	–	+	++	+++
Tannins	–	–	+	++
Glycosides	–	+	+	++
Saponins	–	–	+	++
Steroids	+	+	+	+
Terpenoids	+	++	++	++
Carbohydrates	–	–	+	++
Proteins	–	–	+	++

(– = Absent; + = Present; ++ = Moderately present; +++ = Abundantly present)

Acute Oral Toxicity Study: Acute oral toxicity studies conducted according to OECD Guideline 423 demonstrated that the ethanolic root extract of *Nigella sativa* was well tolerated in experimental animals. No mortality, behavioral abnormalities, or toxic manifestations were observed following oral administration of either 300 or 2000 mg/kg body weight. Based on these findings, 200 mg/kg and 400 mg/kg were selected as the experimental doses for evaluation of wound healing activity.

Evaluation of Wound Healing Activity

Excision Wound Model: Topical treatment with the ethanolic root extract significantly accelerated wound contraction compared with the untreated control group throughout the experimental period. Progressive reduction in wound area was observed from Day 4 onwards in both extract-treated groups. The high-dose group (400 mg/kg) exhibited a faster rate of wound closure than the low-dose group and produced wound contraction values comparable to those obtained with the standard Povidone-Iodine treatment. Complete wound closure was achieved by Day 20 in both the standard and high-dose groups, whereas the control animals still exhibited incomplete healing. Statistical analysis demonstrated significant improvement ($P < 0.05$) in wound contraction in all treated groups compared with the control.

Table 3: Effect of ethanolic root extract on percentage wound contraction.

Group	Day 4	Day 8	Day 12	Day 16	Day 20
Control	18.25 ± 1.45	39.62 ± 2.14	58.73 ± 1.89	78.84 ± 2.12	92.11 ± 1.74
Standard	29.84 ± 1.78*	61.52 ± 1.54*	84.46 ± 1.67*	97.12 ± 1.43*	100
Low dose	24.38 ± 1.58*	52.64 ± 2.07*	76.81 ± 1.91*	91.44 ± 1.62*	98.24 ± 1.26
High dose	27.64 ± 1.42*	57.82 ± 1.76*	81.25 ± 1.72*	95.76 ± 1.38*	100

**Values are Mean ± SEM (n = 6);* $P < 0.05$ versus control.

Effect on Epithelialization Period: The effect of the ethanolic root extract of *Nigella sativa* on epithelialization period is presented in Table 4. Animals treated with the extract exhibited significantly faster epithelialization than the untreated control group ($P < 0.05$). The standard treatment showed the shortest epithelialization period (15.44 ± 0.42 days), followed closely by the high-dose extract group (15.98 ± 0.48 days). The low-dose group also demonstrated a significant reduction in epithelialization time (17.26 ± 0.53 days) compared with the control group (22.82 ± 0.74 days). These findings indicate that the ethanolic extract accelerated re-epithelialization and tissue regeneration in a dose-dependent manner.

Table 4. Effect of *Nigella sativa* Root Extract on Epithelialization Period

Treatment Group	Epithelialization Period (Days)
Control	22.82 ± 0.74
Standard (Povidone-Iodine)	15.44 ± 0.42*
Low Dose (200 mg/kg)	17.26 ± 0.53*
High Dose (400 mg/kg)	15.98 ± 0.48*

**Values expressed as Mean ± SEM (n = 6);* $P < 0.05$ versus control.

Effect on Complete Wound Healing: The duration required for complete wound closure was significantly reduced in all treatment groups compared with the untreated control.

Animals receiving the high-dose ethanolic extract showed complete healing within 15.82 ± 0.43 days, which was comparable to the standard drug (15.24 ± 0.46 days). In contrast, untreated animals required 21.84 ± 0.82 days for complete wound closure. These findings demonstrate that treatment with *Nigella sativa* root extract effectively shortened the overall healing period.

Table 5: Time Required for Complete Wound Healing.

Treatment Group	Healing Time (Days)
Control	21.84 ± 0.82
Standard (Povidone-Iodine)	15.24 ± 0.46*
Low Dose (200 mg/kg)	17.11 ± 0.58*
High Dose (400 mg/kg)	15.82 ± 0.43*

**Values expressed as Mean ± SEM (n = 6);* P < 0.05 versus control.

Incision Wound Model

Effect on Wound Breaking Strength: The wound breaking strength of healed skin is an important indicator of collagen maturation and tissue remodeling. Administration of the ethanolic root extract significantly increased wound breaking strength compared with the control group (P < 0.05). The standard-treated animals exhibited the highest tensile strength (542.62 ± 12.74 g), while the high-dose extract group showed a comparable value (521.14 ± 11.21 g). The low-dose group also demonstrated a significant improvement (472.38 ± 10.56 g) over the control group (342.16 ± 11.48 g). The increase in tensile strength indicates enhanced collagen deposition, better collagen fiber organization, and improved structural integrity of the healed tissue.

Table 6: Effect of *Nigella sativa* Root Extract on Wound Breaking Strength.

Treatment Group	Wound Breaking Strength (g)
Control	342.16 ± 11.48
Standard (Povidone-Iodine)	542.62 ± 12.74*
Low Dose (200 mg/kg)	472.38 ± 10.56*
High Dose (400 mg/kg)	521.14 ± 11.21*

*Values expressed as Mean ± SEM (n = 6); *P < 0.05 versus control.*

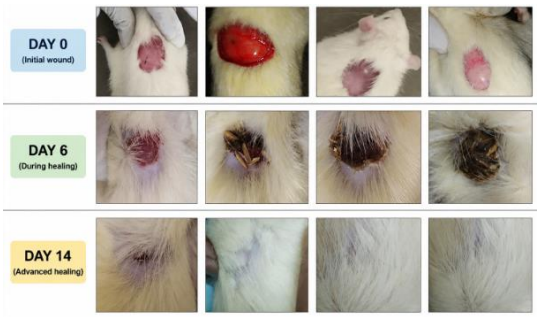

Figure: Wound Healing Process in Rat Model

Histopathological Evaluation: Histopathological examination revealed marked differences between the control and treated groups. Tissue sections from the control group exhibited incomplete epithelialization, sparse collagen fibers, poor granulation tissue formation, minimal angiogenesis, and moderate inflammatory cell infiltration. In contrast, the standard-treated group demonstrated complete epithelialization with dense collagen bundles, extensive fibroblast proliferation, well-developed granulation tissue, prominent angiogenesis, and markedly reduced inflammatory cells. Animals treated with the low-dose extract showed moderate collagen deposition, fibroblast proliferation, improved granulation tissue formation, and only mild inflammatory infiltration. The high-dose extract group exhibited almost complete restoration of normal skin architecture with dense collagen bundles, extensive fibroblast proliferation, complete epithelialization, marked angiogenesis, and minimal inflammatory cell infiltration. These microscopic findings clearly support the gross wound healing observations and indicate that the ethanolic extract promoted all major phases of tissue repair.

Table 7: Histopathological Findings in Different Experimental Groups.

Histopathological Parameter	Control	Standard	Low Dose	High Dose
Epithelialization	+	+++	++	+++
Fibroblast proliferation	+	+++	++	+++
Collagen deposition	+	+++	++	+++
Angiogenesis	+	+++	++	+++
Inflammatory cell infiltration	+++	+	++	+
Granulation tissue formation	+	+++	++	+++

(+ = Mild; ++ = Moderate; +++ = Marked)

Summary

The ethanolic root extract of *Nigella sativa* demonstrated significant wound healing activity in experimental rats. Treatment accelerated wound contraction, reduced epithelialization and complete healing time, and significantly enhanced wound breaking strength. Histopathological evaluation confirmed increased collagen deposition, fibroblast proliferation, angiogenesis, granulation tissue formation, and complete epithelialization with minimal inflammatory cell infiltration. The wound healing response was dose dependent, with the high-dose extract exhibiting efficacy comparable to the standard Povidone-Iodine treatment. Collectively, these findings indicate that the ethanolic root extract possesses promising tissue regenerative potential and supports its traditional use as a natural wound healing agent.

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