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ORIGINAL ARTICLE

Evaluation of the Wound Healing Effect of Topical and Oral Trigonella Foenum-Graecum Seed Oil on Burn Wound in an Animal Model

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Abstract

Background: Burn injuries cause substantial morbidity due to inflammation, infection, and delayed healing. Although silver sulfadiazine (SSD) is widely used for partial-thickness burns, its drawbacks and the lack of a universal standard highlight the need for alternatives. Fenugreek (*Trigonella foenum-graecum*) seed oil has anti-inflammatory, antioxidant, antimicrobial, and pro-healing effects, and may also improve glycemic control—an added benefit for patients with metabolic impairment. **Aims and Objectives:** This study aimed to compare the wound-healing efficacy of topical and oral fenugreek seed oil with SSD in Wistar rats with partial-thickness burns. Primary objectives included assessing wound contraction and histopathological healing. Secondary objectives included evaluating fasting blood glucose levels to determine systemic effects. **Methodology:** Thirty healthy male Wistar rats were randomly divided into five groups (n=6): control (saline), SSD 0.01% ointment, topical fenugreek oil, oral fenugreek oil (1 ml/kg/day), and combined topical plus oral fenugreek oil. Partial-thickness burns were created using a molten-wax model. Treatments were given twice daily for 21 days or until full epithelialization. Wound area was measured on days 4, 9, 14, 18, and 21 to determine contraction. Fasting glucose was assessed on days 1 and 21. Histology evaluated angiogenesis, inflammation, collagen formation, and epithelial repair. **Results:** All groups showed progressive wound contraction, with Groups C (topical fenugreek), D (oral fenugreek), and E (combined therapy) demonstrating significantly faster healing ($p<0.001$ from day 14). Group E achieved complete wound healing in all animals by day 21. Blood glucose levels significantly decreased in Groups A, B, D, and E ($p<0.05$), with the greatest reductions in D and E. Histopathology confirmed reduced inflammation, organized collagen, and complete epithelial regeneration in Groups C, D, and E, with Group E showing the most consistent results. **Conclusion:** Fenugreek seed oil, particularly when administered both topically and orally, significantly improved wound contraction, histological healing, and glycemic control in partial-thickness burn injuries in rats. These findings support its potential as a multifunctional adjunct or alternative to SSD, especially in patients with metabolic disorders. Further clinical studies are recommended to validate efficacy, optimize dosing, and ensure safety in human burn management.

Keywords: Burn wound healing, *Trigonella foenum-graecum*, Fenugreek seed oil, Silver sulfadiazine, Wound contraction, Histopathology

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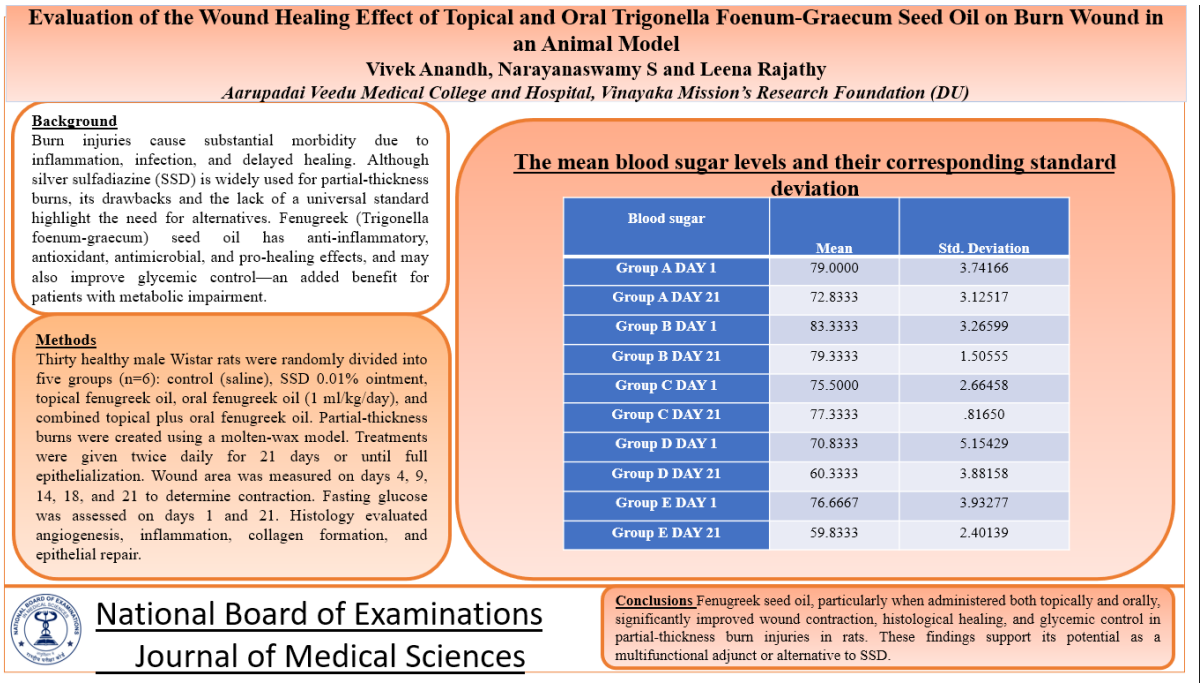
Graphical Abstract



Introduction

Rats are commonly used in burn studies because they are inexpensive and easy to manage, but their healing pattern differs markedly from humans. Human wounds close mainly through re-epithelialization, whereas rats heal largely by contraction due to the panniculus carnosus—a subcutaneous muscle humans lack—which accelerates collagen deposition and wound shrinkage. As a result, rats show faster closure times and rarely develop systemic complications such as sepsis or immunosuppression, which are more typical in larger animal models. These differences make rats useful for rapid mechanistic research but limit how directly the results translate to human burn healing [1–7].

Burns are skin injuries caused by exposure to high heat or caustic chemicals, with thermal and chemical sources being the most common [8]. They are a critical care challenge due to their association with inflammation, tissue destruction, infection, and in severe cases, mortality and long-term disability [9–11]. Burn wounds are classified based on depth: superficial (first-degree), partial-thickness (second-degree), and full-thickness (third-degree) [12–14]. Superficial burns affect only the epidermis and usually heal within five days, whereas partial-thickness burns extend into the dermis and are further divided into superficial and deep partial-thickness types [15–17]. Superficial partial-thickness burns are red, blistered, and very painful, typically healing within 1–3 weeks without surgery, whereas deep partial-thickness burns extend deeper into the dermis, appear dry or dull red, and heal more slowly, often with scarring. Full-thickness burns affect all skin layers and typically require surgical intervention like skin grafting.

Treatment of partial-thickness burns often involves topical agents such as silver sulfadiazine (SSD), silver-impregnated foam dressings, and zinc hyaluronan gels [18–20]. SSD has been the primary agent due to its antibacterial properties, though it

can cause systemic side effects such as methemoglobinemia, neutropenia, and crystalluria [5–7]. There is currently no universally accepted gold standard for treating partial-thickness burns; treatment decisions are typically based on clinician experience and institutional protocols. The lack of controlled comparative studies across various dressings and healing stages limits the strength of evidence supporting any single modality. Additionally, pediatric treatments must consider the cognitive risks of repeated anesthesia during dressing changes. Oxidative stress—through the action of oxygen free radicals—is a known inhibitor of wound healing. In this context, fenugreek (Trigonella foenum graecum), a herb traditionally used for its antidiabetic and digestive benefits, is gaining attention due to its anti-inflammatory, antioxidant, antimicrobial, and wound-healing properties [23–27]. It shows promise in promoting fibro-connective tissue regeneration and organized epidermal healing in burn wounds.

Aim

To assess and contrast the effectiveness of oral and topical Trigonella foenum graecum seed oil with silver sulfadiazine in wound healing in order to determine which is superior. This study sought to determine how SSD and topical and oral Trigonella foenum graecum seed oil affected the rates of histological and clinical healing of skin burn lesions using a rat model.

Objectives

Primary Objective

In animal models of burns, to evaluate the ability of topical and oral Trigonella foenum graecum seed oil to improve wound healing.

Secondary Objectives:

- To assess the wound healing by calculating wound contraction
- To evaluate the histopathological examination of healed wounds for a better conclusion

Materials and Methods

Wistar albino rats were used as study subjects in this experimental animal investigation, which was carried out at the Central Animal House, AVMC. Thirty male Wistar rats in good health were chosen according to certain inclusion criteria, such as having no prior skin lesions or diseases. The study did not include female rats or any ill animals. With a significance level (α) of 5%, an effect size of 1.0, and a statistical power of 90%, the sample size of 30 was determined using G*Power version 3.1 software [28]. Using a basic random sample procedure, the rats were split into five groups of six animals each. To ensure impartial distribution, group allocation was carried out using the GraphPad online random number generator.

Animals were obtained from Biogen Laboratory Animal Facility in Bangalore after receiving consent from IRC and IAEC. They were kept in an animal house at a temperature of 23±3ºC for seven days. Blood samples were sent for biochemical analyses, and animals are rigorously inspected for any health problems and other co-morbidities. On the fourth day, mononuclear cell infiltration and necrotic tissue-based scab formation were evident in all groups. In addition to inflammatory cells penetrating beneath the scab, early attempts at epidermal regeneration were observed. The dermis included hyperemic arteries, but there were no hair, sweat, or sebaceous follicles. After shaving the rat's dorsal surface, the skin

underneath was cleaned with 70% ethanol. Partial thickness burn wound models were employed [28].

A 2-cm metal cylinder filled with 80 °C molten wax was applied to create partial-thickness burns. Each rat was positioned on its back for 10 seconds during the procedure. Wound size was traced on transparent paper on days 4, 9, 14, 18, and 21, then transferred to 1 mm^2 graph paper to calculate the wound area.

Initial wound size minus the size of the wound on a certain day / initial wound size x 100 is the percentage of wound contraction.

Wound healing progresses through four overlapping stages: hemostasis, inflammation, proliferation, and remodeling. Disruptions in any phase can either delay or exaggerate repair. Prolonged inflammation—common in conditions like type 2 diabetes and peripheral vascular disease—slows healing, while excessive tissue growth can lead to hypertrophic scars or keloids.

Group Allocations

Healthy Wistar albino rats (200–250 g) of the Male sex were used for the experiment.

Six animals in each group.

- Group I: Control (normal saline).
- Group II: Topical ointment silver sulfadiazine 0.01% (standard).
- Group III: Topical trigonella foenum graecum seed Oil
- Group IV: Trigonella foenum graecum seed Oil (oral) @ 1 ml/kg body weight/animal
- Group V: Topical Trigonella foenum graecum seed oil + Trigonella foenum graecum seed Oil (oral) @ 1 ml/kg body weight/animal

Study duration: 30 days

All medications were administered every day for 21 days, or until full epithelization, whichever came first. The applications were completed twice a day during the follow-up period. Following the therapy, all of the animals were killed after 21 days of research. Samples of the rats' healed skin were taken for histological examination, and blood was drawn for biochemical examination.

Biochemical investigation

All animals were prepared for the collection of blood samples by warming their tails with a hot water cloth for around five minutes in order to promote vasodilation. In order to reduce the danger of infection, the rats were then gently restrained and their tails were cleaned using alcohol swabs. After locating the lateral tail vein, a 23–25 gauge needle with the bevel facing up was cautiously inserted at a shallow angle. A syringe's suction was used to pull blood into a collection tube. Following collection, a sterile cotton swab was used to gently press until the bleeding stopped. After being collected, the blood samples were placed in vacutainers and submitted for analysis, which included measuring the fasting blood glucose levels [29, 30, 31].

Histopathological Examination

On day 21, rats were put to sleep, and their healed skin samples were removed and placed in a 10% buffered formalin solution. The samples underwent standard histopathology procedures before being embedded in paraffin blocks. Haematoxylin and eosin staining was used to assess the slices (5 μm) from the blocks under a light microscope for angiogenesis, inflammatory cell infiltration, collagen

buildup and granulation tissue, and epithelialization.

Statistical Methods

For statistical analysis, the students' t-test and one-way analysis of variance (ANOVA) were employed. A value of $P < 0.05$ was considered statistically significant (Table 1).

Table 1. Dose of Ketamine and Xylazine calculated exactly to each rat's body weight

Rat b.w	**Xylazine (10 mg/kg b.w)**	**Ketamine (80 mg/kg b.w)**
150 g	0.06 ml	0.24 ml
200 g	0.08 ml	0.32 ml
250 g	0.13 ml	0.40 ml
300 g	0.15 ml	0.48 ml
350 g	0.18 ml	0.56 ml
400 g	0.20 ml	0.64 ml
450 g	0.23 ml	0.72 ml

(b.w. - Body weight); Xylazine: 1 ml = 23.32 mg; Ketamine: 1 ml = 50 mg

Results

Table 2. Wound size distribution of the five test Groups on days 4, 9, 14, 18 and 21

Group	Animal No.	4th Day	9th Day	14th Day	18th Day	21st Day
A	1	2.1	1.8	1.6	1.1	0.5
A	2	2.1	1.8	1.6	0.9	0.5
A	3	2.2	1.7	1.5	1	0.5
A	4	2	1.8	1.4	0.8	0.4
A	5	1.9	1.5	1.3	0.8	0.4
A	6	2	1.9	1.5	0.7	0.4
B	1	2	1.6	1.2	0.6	0.2

B	2	2.3	1.7	1.2	0.5	0
B	3	2.1	1.8	1.1	0.8	0
B	4	2.1	1.7	1.1	0.8	0
B	5	2.2	1.6	0.9	0.7	0
B	6	2.1	1.7	0.9	0.6	0.1
C	1	2.2	1.8	1.1	0.3	0
C	2	2.1	1.7	1.1	0.5	0.2
C	3	2.1	1.6	0.9	0.3	0
C	4	2	1.8	1	0.5	0.2
C	5	2	1.7	1	0.6	0
C	6	1.9	1.5	1	0.5	0.2
D	1	2.2	1.8	1	0.8	0.2
D	2	2	1.6	1	0.8	0
D	3	2.1	1.7	1	0.7	0.2
D	4	1.9	1.5	0.9	0.6	0
D	5	2.2	1.6	0.9	0.6	0.2
D	6	2	1.5	0.8	0.5	0
E	1	2.1	1.6	0.9	0.7	0.1
E	2	2	1.6	0.9	0.6	0
E	3	1.9	1.4	1	0.6	0
E	4	1.8	1.3	0.8	0.5	0
E	5	2.1	1.5	0.9	0.5	0
E	6	2.2	1.6	0.8	0.4	0.1

The Table 2 shows across all groups (A–E), a consistent reduction in wound size over time was observed, indicating progressive healing. In Group A, wound sizes decreased from 1.9–2.2 cm on the 4th day to 1.5–1.9 cm by the 9th day, 1.3–1.6 cm on the 14th day, 0.7–1.1 cm on the 18th day, and finally 0.4–0.5 cm by the 21st day. Group B showed a similar trend, starting at 2.0–2.3 cm on day 4, reducing to 1.6–1.8 cm by day 9, 0.9–1.2 cm on day 14, and eventually reaching 0.5–0.8 cm on day 18 and 0.0–0.2 cm by day 21. Group C began with wound sizes of 1.9–2.2 cm on day 4, which declined to 1.5–1.8 cm by day 9, 0.9–1.1 cm by day 14, 0.3–0.6 cm on day 18, and 0.0–0.2 cm by day 21. Group D also demonstrated steady healing, with initial sizes of 1.9–2.2 cm on day 4, followed by 1.5–1.8 cm on day 9, 0.8–1.0 cm on day 14, 0.5–0.8 cm on day 18, and 0.0–0.2 cm by day 21. Lastly, Group E started with wound sizes of 1.8–2.2 cm on day 4, reduced to 1.3–1.6 cm on day 9, 0.8–1.0 cm on day 14, 0.4–0.7 cm on day 18, and finally decreased to 0.0–0.1 cm by day 21. These results collectively demonstrate effective wound healing across all experimental groups over the 21-day observation period (Table 3).

Table 3. The mean wound contraction values and their corresponding standard deviations at different time points (4th, 9th, 14th, 18th, and 21st day) for all the Groups

	Groupson Day 4	Groups on Day 9	Groupson Day14	Groupson Day 18	Groupson Day 21
Mean	2.0633	1.6467	1.0767	.6433	.1467
Std. Deviation	.11592	.13830	.23589	.18696	.17564

The data illustrates a steady decrease in mean wound contraction values over time, starting from 2.0633 on the 4th day and progressively reducing to 0.1467 by the 21st day, indicating consistent wound healing across all groups. The standard deviation, which reflects the variability in wound contraction measurements, begins at a relatively low value of 0.11592 on the 4th day, suggesting minimal variation among samples at the outset. It slightly increases to 0.13830 on the 9th day and rises further to 0.23589 on the 14th day, showing greater variability during the middle phase of healing. This variability peaks at 0.18696 on the 18th day before slightly declining to 0.17564 by the 21st day. Overall, the data reflects a clear trend of decreasing wound size over time, accompanied by moderate fluctuations in variability, particularly during the intermediate healing stages, suggesting a generally consistent and effective healing process (Table 4).

Table 4. The ANOVA results for wound contraction at different time points (9th, 14th, 18th, and 21st days)

ANOVA for wound contraction at different time points						
Day		Sum of Squares	df	Mean Square	F	Sig.
9TH	Between Groups	409.272	4	102.318	4.327	.009
	Within Group	591.136	25	23.645		
	Total	1000.408	29			
14TH	Between Groups	3194.075	4	798.519	33.142	<.001
	Within Group	602.347	25	24.094		
	Total	3796.423	29			
18TH	Between Groups	1359.700	4	339.925	9.897	<.001
	Within Group	858.623	25	34.345		
	Total	2218.322	29			
21ST	Between Groups	2075.444	4	518.861	25.649	<.001
	Within Group	505.733	25	20.229		
	Total	2581.177	29			

ANOVA results show significant differences in wound contraction between groups at all time points from the 9th to the 21st day. On the 9th day, a p-value of 0.009 and F-value of 4.327 indicate statistical significance. By the 14th day, the differences are highly significant with a p-value < 0.001 and F-value of 33.142. Similarly, on the 18th day, a p-value < 0.001 and F-value of 9.897 confirm significant group differences. On the 21st day, very high significance is observed with a p-value < 0.001 and F-value of 25.649 (Table 5).

Table 5. The mean blood sugar levels and their corresponding standard deviations for five Groups (A, B, C, D, and E) measured on Day 1 and Day 21

Blood sugar	**Mean**	**Std. Deviation**
Group A DAY 1	79.0000	3.74166
Group A DAY 21	72.8333	3.12517
Group B DAY 1	83.3333	3.26599
Group B DAY 21	79.3333	1.50555
Group C DAY 1	75.5000	2.66458
Group C DAY 21	77.3333	.81650
Group D DAY 1	70.8333	5.15429
Group D DAY 21	60.3333	3.88158
Group E DAY 1	76.6667	3.93277
Group E DAY 21	59.8333	2.40139

The data presents the mean blood sugar levels and corresponding standard deviations for Groups A to E on Day 1 and Day 21. On Day 1, Group A had a mean of 79.00 (SD: 3.74), Group B 83.33 (SD: 3.27), Group C 75.50 (SD: 2.66), Group D 70.83 (SD: 5.15), and Group E 76.67 (SD: 3.93), showing varying degrees of baseline blood sugar and variability. By Day 21, most groups exhibited decreased blood sugar levels: Group A dropped to 72.83 (SD: 3.13), Group B to 79.33 (SD: 1.51), Group D to 60.33 (SD: 3.88), and Group E to 59.83 (SD: 2.40), all indicating reductions with improved consistency. Group C, however, showed a slight increase to 77.33 (SD: 0.82), but with the least variability among all groups. In summary, Groups D and E demonstrated the most significant decreases in blood sugar, while Groups A and B had moderate reductions, and Group C showed a minor increase with minimal variation, indicating an overall trend of blood sugar reduction with differing consistency across groups (Table 6).

Table 6. The t-test results & p-values in blood sugar levels between Day 1 and Day 21

Blood sugar	t-test	p value
Group A DAY 1 - DAY 21	5.89	0.00
Group B DAY 1 - DAY 21	3.10	0.01
Group C DAY 1 - DAY 21	-1.81	0.07
Group D DAY 1 - DAY 21	10.97	0.00
Group E DAY 1 - DAY 21	7.18	0.00

The paired t-test showed changes in blood glucose from Day 1 to Day 21 for each group. Groups A and B demonstrated significant reductions (A: $t = 5.89$, $p = 0.00$; B: $t = 3.10$, $p = 0.01$). Group C showed no meaningful change ($t = 1.81$, $p = 0.07$). Group D had a marked and highly significant decrease ($t = 10.97$, $p = 0.00$). Group E also showed a significant reduction ($t = 7.18$, $p = 0.001$).

In conclusion, blood sugar levels in Groups A, B, D, and E significantly decreased, whilst Group C showed no discernible change. This implies that most groups' blood sugar levels were more significantly impacted by the intervention or time effect, with Group D and Group E seeing the biggest drops.

Histopathological Examination

Excised wound tissues were examined histologically. In Group A, animals 1, 2, and 6 showed healing, while 3–5 displayed inflammatory changes with no repair. In Group B, animals 1, 2, and 5 healed, animals 3 and 4 showed fibrinopurulent exudate, and animal 6 had dense neutrophilic and lymphocytic infiltration without healing. In Group C, all animals except number 4 showed healing. In Group D, animals 1–4 and 6 healed, while animal 5 had an ulcer with no repair. In Group E, all six animals showed complete healing. Overall, Groups C, D, and E demonstrated markedly better wound healing than Groups A and B.

Discussion

The current experimental study explored the efficacy of various interventions on wound contraction and blood glucose regulation in Wistar rats over a 21-day period. Across all five groups (A–E), a progressive reduction in wound size was observed, with Groups C, D, and E demonstrating the most rapid and complete healing by the 21st day. This indicates that the applied treatments, particularly in the latter three groups, facilitated wound closure more effectively. Such progressive wound contraction is typically attributed to decreased inflammation, increased fibroblast activity, collagen deposition, and angiogenesis—key components of the proliferative and remodeling phases of wound healing [32].

Quantitative analysis using ANOVA further confirmed the significant differences in wound healing across groups, especially from Day 9 onwards. The differences became highly significant by Day 14 ($p < 0.001$), indicating that the

interventions started exhibiting maximum efficacy during this intermediate healing phase. These findings align with those of Bakhtiari et al., who noted that plant-based therapies often show delayed but sustained wound-healing effects due to their action on modulating cytokine responses and enhancing granulation tissue formation [33]. Additionally, the reduced wound size in Groups D and E correlates with literature supporting the use of natural agents—such as fenugreek or silver-based formulations—for their antimicrobial, antioxidant, and anti-inflammatory actions [34].

The mean wound contraction data also demonstrated a consistent downward trend from Day 4 (mean 2.06 cm) to Day 21 (mean 0.14 cm). This progressive reduction, accompanied by slight variations in standard deviation over time, reflects the uniformity and reliability of the healing process across different groups. Variability peaked slightly during the proliferative phase (Day 14–18), which could correspond to individual biological responses to interventions. This observation is supported by Eming et al., who noted that variability in healing responses often arises during tissue remodeling when fibroblasts, keratinocytes, and immune cells are actively proliferating and migrating [35].

Importantly, the interventions also impacted systemic physiology, as seen in the fasting blood glucose levels. Groups D and E exhibited the most significant reductions in blood glucose by Day 21, which may suggest the antidiabetic potential of the treatments used in these groups. Trigonella foenum-graecum (fenugreek), in particular, has been widely studied for its glucose-lowering properties through mechanisms involving improved insulin sensitivity, delayed carbohydrate digestion, and enhanced peripheral glucose uptake [36]. The combination of glycemic control and accelerated wound healing is particularly valuable, especially in diabetic populations where chronic hyperglycemia impairs angiogenesis, collagen deposition, and immune function, thereby delaying wound closure [37].

T-test analysis confirmed statistically significant blood glucose reductions in Groups A, B, D, and E ($p < 0.05$), with the most prominent effects in Group D ($t = 10.97$) and Group E ($t = 7.18$). In contrast, Group C showed a slight, non-significant increase in glucose levels, possibly indicating a lack of systemic metabolic effect despite good wound healing. This could suggest that the intervention in Group C was locally effective on wound sites but had limited systemic influence. Such divergence is consistent with studies by Ahmad et al., which demonstrated that not all phytochemicals produce both local and systemic benefits, and their bioavailability and pharmacokinetics can vary considerably [38].

Histopathological findings further corroborated the quantitative results. Complete wound healing was noted in all animals of Group E, followed by significant healing in most animals of Groups C and D. In contrast, inflammatory markers and fibrinopurulent exudates were frequently observed in Groups A and B, indicating incomplete or delayed healing. The presence of neutrophilic and lymphocytic infiltration in these groups suggests persistent inflammation, which can hinder epithelial regeneration and extracellular matrix remodeling. These results mirror those of Sharma et al., who highlighted the importance of timely resolution of

inflammation in ensuring efficient wound healing [39].

Conclusion

This experimental study demonstrated that *Trigonella foenum-graecum* (fenugreek) seed oil, especially when administered both topically and orally (Group E), significantly enhanced wound contraction, histological healing, and blood glucose reduction in Wistar rats with partial-thickness burn injuries. Groups C, D, and E achieved faster and more complete wound closure compared to the control (Group A) and silver sulfadiazine-treated group (Group B), with Group E showing complete healing in all animals. The dual benefits of local wound repair and systemic glycemic control, particularly in Groups D and E, highlight the potential of fenugreek as a multifunctional therapeutic agent for burn management, especially in metabolically compromised states. Histopathology confirmed reduced inflammation, organized collagen deposition, and epithelial regeneration in these groups, supporting the observed quantitative outcomes. These findings suggest that fenugreek oil—alone or in combination with systemic administration—may be a valuable adjunct or alternative to conventional burn treatments.

Statements and Declarations

Conflicts of interest

The authors declare that they do not have conflict of interest.

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