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Assessment of the anti-inflammatory, antioxidant, and wound-healing effects of methanolic soybean seed extract in an excision wound model in albino rats

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ABSTRACT

Wound healing is a complex physiological process influenced by oxidative stress and inflammation. This study assessed the antioxidant, anti-inflammatory, and wound-healing effects of a methanolic extract of soybean seeds using a full-thickness excision wound model in male albino rats. Fourteen rats were randomly divided into two groups (n = 7). Under anesthesia, full-thickness skin wounds were aseptically created in the thoraco-abdominal region. Group A received sterile water (placebo) topically, while Group B received the soybean seed extract daily for 21 days. Wound healing was evaluated by macroscopic examination, measurement of wound contraction, and analysis of inflammatory and oxidative stress markers.

By day 7, wound contraction was significantly higher in the control group (60.42 ± 6.65%) compared to the extract-treated group (43.96 ± 11.58%) ($p < 0.05$). No significant difference was observed in the neutrophil-to-lymphocyte ratio between the two groups. However, biochemical analyses showed elevated levels of serum malondialdehyde (MDA) and superoxide dismutase (SOD) activity in the treated group (MDA: 4.26 ± 0.39 µmol/mg; SOD: 1.43 ± 0.16 mg/mL) versus the control (MDA: 3.18 ± 0.51 µmol/mg; SOD: 1.01 ± 0.13 mg/mL) ($p < 0.05$).

In conclusion, topical application of soybean seed methanolic extract did not enhance wound healing but improved antioxidant markers, indicating its potential role in mitigating oxidative stress rather than directly accelerating tissue repair.

Keywords: antioxidant, oxidative stress, management, ethnopharmacology, ethnoveterinary medicine.

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Evaluación de los efectos antiinflamatorios, antioxidantes y cicatrizantes del extracto metanólico de semillas de soja en un modelo de herida por escisión en rata albina

RESUMEN

La cicatrización de heridas es un proceso fisiológico complejo influenciado por el estrés oxidativo y la inflamación. Este estudio evaluó los efectos antioxidantes, antiinflamatorios y cicatrizantes de un extracto metanólico de semillas de soya utilizando un modelo de herida por escisión de espesor completo en ratas albinas machos. Catorce ratas fueron divididas aleatoriamente en dos grupos ($n = 7$). Bajo anestesia, se indujeron heridas cutáneas de espesor completo de forma aséptica en la región toracoabdominal. El Grupo A recibió agua estéril tópica, mientras que el Grupo B fue tratado diariamente con el extracto de semillas de soya durante 21 días. La cicatrización fue evaluada mediante examen macroscópico, medición de la contracción de la herida y análisis de marcadores inflamatorios y de estrés oxidativo.

Para el día 7, la contracción de la herida fue significativamente mayor en el grupo control (60.42 ± 6.65%) en comparación con el grupo tratado con el extracto (43.96 ± 11.58%) ($p < 0.05$). No se observaron diferencias significativas en la relación neutrófilo-linfocito entre los grupos. Sin embargo, los análisis bioquímicos mostraron niveles elevados de malondialdehído (MDA) en suero y actividad de superóxido dismutasa (SOD) en el grupo tratado (MDA: 4.26 ± 0.39 μmol/mg; SOD: 1.43 ± 0.16 mg/mL) frente al control (MDA: 3.18 ± 0.51 μmol/mg; SOD: 1.01 ± 0.13 mg/mL) ($p < 0.05$).

En conclusión, la aplicación tópica del extracto metanólico de semillas de soya no mejoró la cicatrización de heridas, pero sí incrementó los marcadores antioxidantes, lo que sugiere un posible papel terapéutico en la mitigación del estrés oxidativo más que en la aceleración directa de la reparación tisular.

Palabras clave: antioxidante, estrés oxidativo, gestión, etnofarmacología, medicina etnoveterinaria.

INTRODUCTION

A cutaneous wound is defined as damage to the epithelial integrity of the skin's outer layer, resulting in the disruption of both the structure and function of the underlying tissue (Verma *et al.*, 2019). Wounds may result from various causes, including physical trauma, external factors (e.g., pressure, burns, and lacerations), or pathological conditions such as diabetes and vascular diseases (Tottoli *et al.*, 2020; Atala *et al.*, 2021). Wound healing (WH) is a complex, multi-phase biological process consisting of hemostasis, inflammation, proliferation, and tissue remodeling (Kilani *et al.*, 2025; Gushiken *et al.*, 2021; Rodrigues *et al.*, 2019). These stages involve inflammatory cells such as neutrophils, lymphocytes, and macrophages, which play key roles in the early phase by removing debris and pathogens to facilitate tissue regeneration (Berman *et al.*, 2017). The proliferative phase of WH includes angiogenesis, fibroblast proliferation, extracellular matrix deposition, and granulation tissue formation, while the remodeling phase is essential for restoring tissue strength (Plikus *et al.*, 2017; Liu *et al.*, 2020).

One of the primary barriers to effective wound healing is the persistence of oxidative stress and prolonged inflammation (Gushiken *et al.*, 2021). While reactive oxygen species (ROS) are vital for host defense and cell signaling at physiological levels, excessive ROS production induces oxidative damage, chronic inflammation, delayed tissue regeneration, and an increased risk of wound chronicity (Dunnill *et al.*, 2015; Sanchez *et al.*, 2018). Despite advances in wound care, managing chronic and complex wounds remains a challenge, largely due to persistent inflammation, oxidative stress, and microbial colonization (Kilani *et al.*, 2025; Gushiken *et al.*, 2021; Atala *et al.*, 2021). These challenges have spurred growing interest in natural antioxidants and bioactive compounds capable of modulating oxidative damage and promoting tissue repair (Mohsin *et al.*, 2022; Rahman *et al.*, 2023). Natural products are increasingly recognized as valuable sources of alternative medicine and bioactive compounds for the treatment of numerous conditions. Medicinal plants and traditional remedies used in wound care are often accessible, affordable, and, in some cases, freely available—particularly in regions with limited access to conventional medical treatments (Agyare *et al.*, 2009).

Soybean (*Glycine max*) seeds are rich in bioactive compounds, including isoflavones (genistein, daidzein), phenolic acids, flavonoids, saponins, and phytosterols (Lee *et al.*, 2008; Messina *et al.*, 2010). Recent studies have demonstrated that these compounds possess potent antioxidant and antimicrobial properties relevant to wound healing (Shen *et al.*, 2020; Kim *et al.*, 2021). Isoflavones, in particular, have been shown to scavenge free radicals, modulate cytokine expression, and enhance fibroblast activity, all of which are critical for tissue repair (Kim *et al.*, 2021; Shen *et al.*, 2020). Furthermore, soybean-derived saponins and phenolic compounds have been reported to stimulate angiogenesis and collagen synthesis, supporting their therapeutic potential in wound management (Zhang *et al.*, 2019).

However, there remains a lack of experimental data on the direct topical wound-healing effects of soybean seed methanolic extract, particularly regarding its impact on oxidative and inflammatory pathways during cutaneous repair. Therefore, this study aimed to evaluate the wound-healing efficacy of soybean seed methanolic extract, as well as its antioxidant and anti-inflammatory effects, using an experimental excision wound model in rats.

MATERIALS AND METHODS

Experimental animals

A preliminary power analysis was conducted to minimize the number of animals used, in alignment with the 3R principles (Replacement, Reduction, Refinement). The sample size was calculated using G*Power (version 3.1.9.7) for a one-way ANOVA (fixed effects, omnibus test), assuming a large effect size ($f = 0.8$), a significance level (α) of 0.05, and a statistical power ($1-\beta$) of 0.80. This analysis yielded a total sample size of fourteen (14) male albino rats. The animals, each weighing approximately 120 g and deemed apparently healthy, were sourced from a laboratory animal facility in Ibadan, Oyo State.

The rats were individually housed in metal cages at the Laboratory Animal Unit of the Veterinary Teaching Hospital, College of Veterinary Medicine (COLVET), Federal University of Agriculture, Abeokuta, Ogun State, where the study was conducted. The animals were allowed a two-week acclimatization period to adapt to the laboratory environment. During this period and throughout the experiment, they were fed *ad libitum* with Growers Mash and had unrestricted access to clean drinking water provided hygienically.

All animals received appropriate care and humane handling in accordance with ethical standards and prior studies involving plant-based wound treatments in similar models (Zhang *et al.*, 2020; Kim *et al.*, 2021). This randomized experimental protocol was approved by the Research Ethics Committee of the College of Veterinary Medicine (COLVET), Federal University of Agriculture, Abeokuta, under approval number FUNAAB/COLVET/CREC/2024/05/02.

Crude extraction of soybean seed and phytochemical analysis

Soybean seeds were purchased from Sabo, Eleweran Market, Ogun State, and taxonomically identified as *Glycine max* (L.) Merr. (Fabaceae) at the Department of Botany, College of Biological Sciences, Federal University of Agriculture, Abeokuta. A voucher specimen was deposited under the number FHA-4337.

The seeds were thoroughly dried, ground into a fine powder, and approximately 650 g of the powder was soaked in 1.2 L of 90% methanol for 72 hours with intermittent stirring to enhance extraction. The resulting mixture was filtered using muslin cloth, and the filtrate was concentrated with a rotary evaporator at 45°C. The final crude extract, weighing approximately 21 g, was stored in an airtight glass container at 4°C until further use.

As shown in table 1, qualitative phytochemical screening was performed using standard tests to detect the presence of various classes of bioactive compounds, including phenolic compounds, flavonoids, saponins, alkaloids, steroids, and carbohydrates. Quantitative phytochemical analysis was also conducted to determine the concentrations of these compounds using established colorimetric and gravimetric methods, as previously described by Hashim *et al.* (2021).

TABLE 1. Phytochemical composition of the methanolic extract of soybean seeds used in the wound healing study

Phytochemicals	Test(s) performed	Soybean methanolic extract	% Yield (%w/w)
Saponins	Frothing test	++	0.80±0.00
Tannins	Ferric chloride test	+	2.23±0.01
Flavonoids	Ammonia/H2SO4 test	++	3.39±0.10
Cardiac glycosides	Keller-Killiani test	+	NA
Anthraquinones	Borntrager's test	+	NA
Terpenoids	Salkowski test	+	0.65±0.00
Steroids	Liebermann-Burchard test	++	NA
Alkaloids	Dragendorff's test	++	8.5±0.00
Phenol	Keller-Killiani test	++	2.07±0.00

++ = Abundant, + = Present, NA= Not Available.

Source: own elaboration.

Experimental wound creation

Prior to wound induction, all animals were fasted for 4 hours. Anesthesia was administered intramuscularly using 80 mg/kg of 5% ketamine hydrochloride (Rotexmedica, Trittau, Germany) and 10 mg/kg of 2% xylazine hydrochloride (Xylased, Bioveta, Ivanovice, Czech Republic). The dorsal thoraco-abdominal region was then aseptically prepared, and a full-thickness excisional skin wound measuring 2 × 2 cm was created on each rat. Following wound creation, the animals were returned to their individual cages lined with paper bedding.

Experimental design and wound management

Fourteen (14) clinically healthy male albino rats were randomly assigned to two groups (n = 7 per group), designated as Group A and Group B. Rats in Group A received a daily topical application of sterile water and served as the control group. Rats in Group B were treated topically with 100% (w/v) methanolic extract of soybean seeds.

WOUND HEALING EVALUATION

Gross wound assessment

Wounds were evaluated macroscopically throughout the study period for granulation tissue development, progression of epithelialization, exudate production, scab formation, wound bed appearance, presence of pus or necrotic tissue, as well as changes in color and odor.

Assessment of wound contraction

Wound contraction, expressed as the percentage of wound closure, was assessed using the wound tracing technique on days 0, 3, 7, 11, and 15. A transparent tracing sheet was gently placed over each wound to delineate the margins, which were then outlined using a fine-tip permanent marker. The traced area was measured using thread and a ruler. The percentage of wound contraction was calculated using Wilson's formula, considering the wound area on day 0 as the baseline (100%) (Chen *et al.*, 2015):

$$\%\ \text{wound contraction} = \frac{Initial\ (0\ day) wound\ area - Wound\ area\ of\ specific\ day}{Initial\ (0\ day)\ wound\ area} \times 100$$

Neutrophil-to-Lymphocyte Ratio (NLR)

On day 9, blood samples were collected from five rats in each group into ethylenediaminetetraacetic acid (EDTA) tubes for neutrophil and lymphocyte counts, which were performed using an automated hematology analyzer (Biobase Auto-Hemoanalyzer, Shandong, China). The neutrophil-to-lymphocyte ratio (NLR) was subsequently calculated for each sample.

Evaluation of oxidative stress and antioxidant markers in serum and granulation tissue

On day 9, blood and granulation tissue samples were collected from five rats per group. Blood was drawn into plain tubes (without anticoagulant), and serum was separated by centrifugation at 15,000 × g for 5 minutes. Following blood collection, granulation tissue was excised from each rat and processed for antioxidant analysis.

Both serum and tissue samples were analyzed for oxidant and antioxidant markers, including superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), and the oxidative stress biomarker malondialdehyde (MDA), using previously established methods (Rahman *et al.*, 2017).

Statistical analysis

The Shapiro–Wilk test was performed to evaluate the normality of data distribution. All variables met the assumption of normality, allowing for parametric analysis. Statistical analysis was conducted using the Statistical Package for the Social Sciences (SPSS), version 25. Results were expressed as mean ± standard deviation (SD). Differences in wound contraction and oxidative/antioxidant parameters between groups were assessed using an independent samples *t*-test. A *p*-value < 0.05 was considered statistically significant.

RESULTS

Qualitative and quantitative phytochemical analysis of soybean seed methanolic extract

The qualitative and quantitative phytochemical screening of the methanolic extract of soybean seeds revealed the presence of several bioactive compounds. These included alkaloids (8.5 ± 0.00 % w/w), flavonoids (3.39 ± 0.10 % w/w), tannins (2.23 ± 0.01 % w/w), phenols (2.07 ± 0.01 % w/w), saponins (0.80 ± 0.00 % w/w), and terpenoids (0.65 ± 0.00 % w/w), as presented in table 1.

Wound healing properties of soybean seed methanolic extract

Wounds in both groups assumed an irregular round shape, with coloration ranging from dark red to brown beginning on day 3 (figure 1). By day 7, a noticeable increase in wound contraction was observed (table 2), with the wound bed in Group

TABLE 2. Percentage of wound contraction measured during experimental wound healing in the male albino rat excision wound model

Days	Group	Mean ± SD (%)	Confidence Interval	*p*- value
Day 3	Group A	27.50±10.00	-3.98-20.48	0.161
	Group B	19.25±7.37		
Day 7	Group A	60.42±6.65	4.32-28.6	0.013*
	Group B	43.96±11.58		
Day 11	Group A	82.50±6.61	-3.44-53.44	0.068
	Group B	57.50±14.14		
Day 15	Group A	95.63±0.00	-254.47-268.22	0.795
	Group B	74.38±16.79		

*Value is significant, statistically at p<0.05

Source: own elaboration.

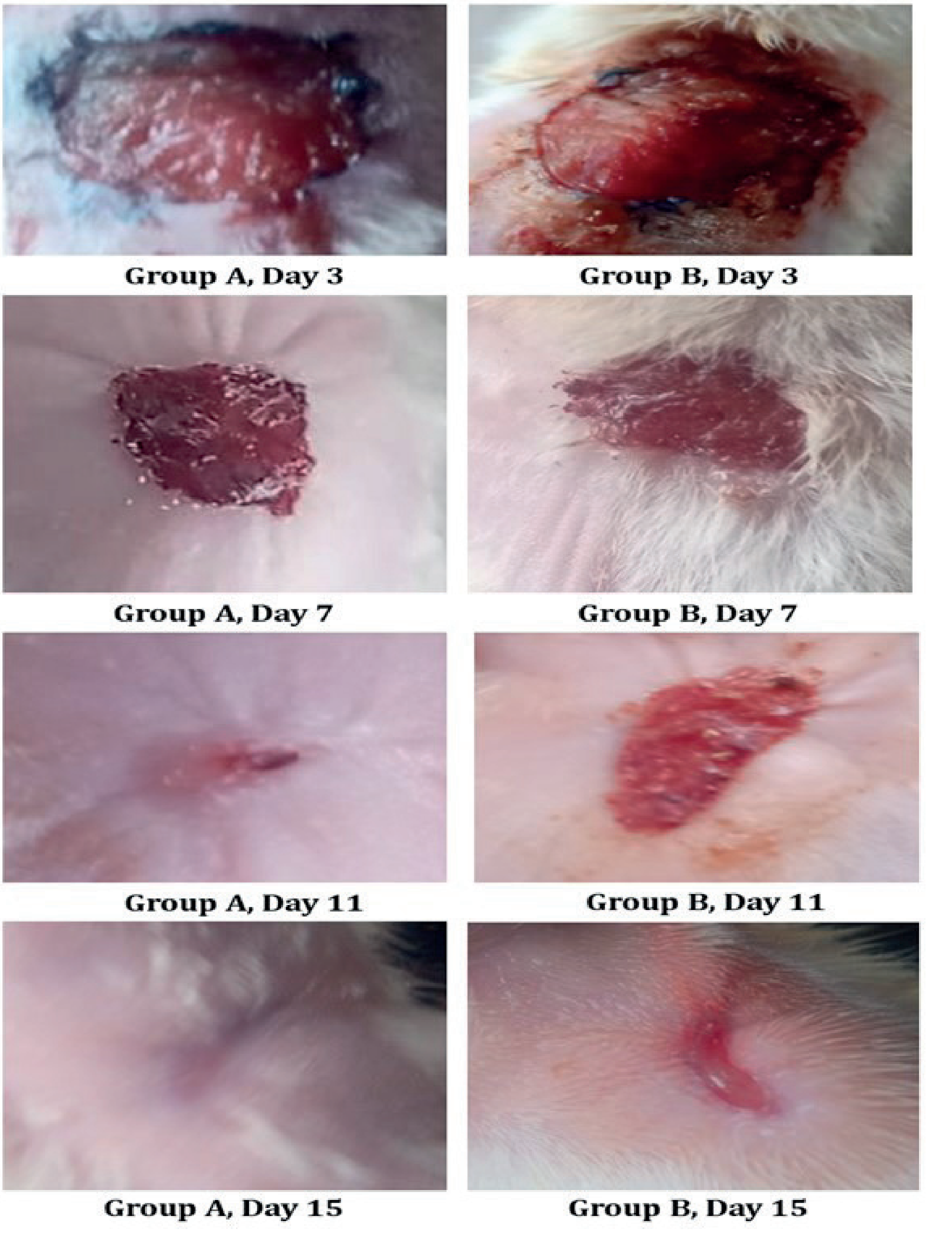

FIGURE 1. Gross appearance of excision wounds and percentage wound contraction in the male albino rat experimental wound healing model. A higher rate of epithelialization and wound contraction was observed on days 7 and 11 in Group A (treated with sterile water) compared to Group B (treated with soybean seed methanolic extract).

Source: own elaboration.

B appearing redder compared to Group A. However, the percentage of wound contraction in Group A (60.42 ± 6.65%) was significantly higher than that in Group B (43.96 ± 11.58%) ($p < 0.05$).

On day 11, wounds in Group A showed a more rapid rate of epithelialization and contraction, with the wound bed covered by a thin scab. Although the percentage of wound contraction in the control group (Group A) reached 82.50 ± 6.61%, compared to 57.50 ± 14.14% in Group B, the difference was not statistically significant.

By day 15, complete healing was observed in Group A, with a wound contraction rate of 95.63 ± 0.00%, whereas wounds in Group B had not yet fully healed, showing a contraction rate of 74.38 ± 16.79%.

Additionally, the neutrophil-to-lymphocyte ratio (NLR) was similar between the groups, with values of 0.55 in Group A and 0.53 in Group B, showing no statistically significant difference (figure 2).

Serum levels of malondialdehyde (MDA) and superoxide dismutase (SOD) (table 3) were significantly higher ($p < 0.05$) in the group treated with soybean seed methanolic extract compared to the group treated with sterile water. However, no significant differences were observed between the groups in serum levels of reduced glutathione (GSH) and catalase (CAT).

Although tissue concentrations of malondialdehyde and superoxide dismutase were not significantly different ($p > 0.05$), their values were higher in Group B than in Group A (table 4). Likewise, tissue levels of reduced glutathione and catalase showed no significant differences ($p > 0.05$) between the two groups.

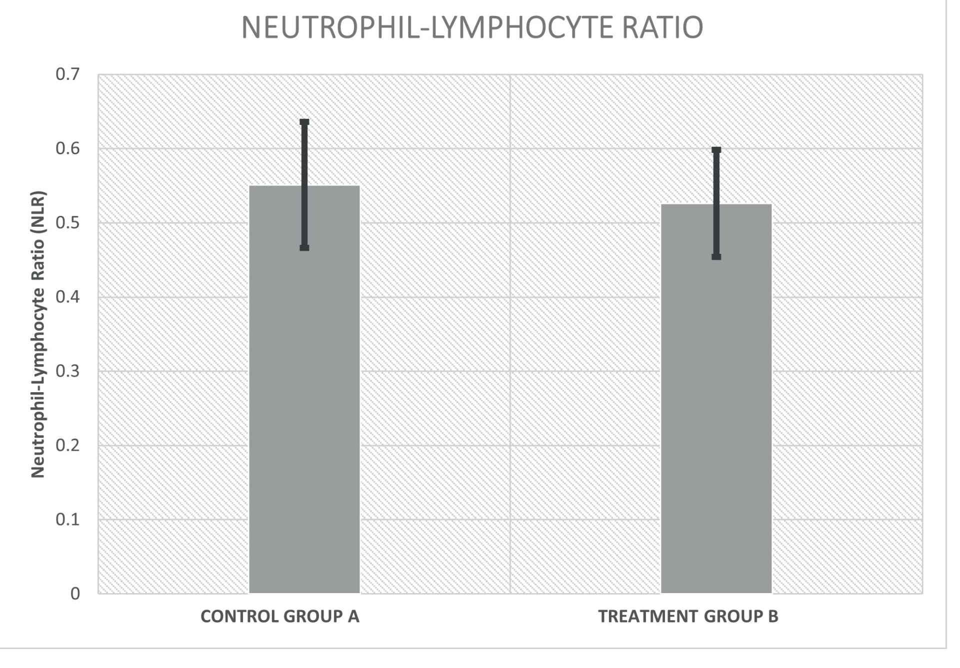

FIGURE 2. Neutrophil-to-lymphocyte ratio in the male albino rat excision wound healing model.

Source: own elaboration.

TABLE 3. Serum oxidant and antioxidant activities during experimental wound healing in rats treated with soybean seed methanolic extract

Parameters	Group	Mean ± SD	*p*- value
MDA (μmols/mg protein)	Group A	3.18± 0.51	0.04*
	Group B	4.26 ± 0.39	
SOD (mg/mL)	Group A	1.01 ± 0.13	0.02*
	Group B	1.43 ± 0.16	
GSH (μg/ml)	Group A	11.83± 0.40	0.979
	Group B	11.80± 1.10	
CAT (μg/ml)	Group A	0.22± 0.05	0.612
	Group B	0.27± 0.13	

*Value is significant, statistically at $p<0.05$

Source: own elaboration.

TABLE 4. Tissue oxidant and antioxidant activities during experimental wound healing in rats treated with soybean seed methanolic extract

Parameters	Group	Mean+SD	*p* -value
MDA (μmols/mg protein)	Group A	1.71± 0.59	0.401
	Group B	2.06± 0.26	
SOD (mg/mL)	Group A.	1.00± 0.25	0.249
	Group B.	1.59± 0.71	
GSH (μg/ml)	Group A	15.37± 0.55	0.160
	Group B	13.10± 2.21	
CAT (μg/ml)	Group A	0.35± 0.13	0.153
	Group B	0.21± 0.06	

Source: own elaboration.

DISCUSSION

The presence of saponins, tannins, flavonoids, cardiac glycosides, anthraquinones, terpenoids, steroids, alkaloids, and phenols in the methanolic extract of soybean seeds used in this study is consistent with previous reports (Hidayat *et al.*, 2018; Kumaran *et al.*, 2015; Prahastuti *et al.*, 2019). Flavonoids were identified as the second most abundant phytochemical class in the extract. This suggests a high content of isoflavones—flavonoid compounds structurally similar to endogenous 17β-estradiol—known for their diverse pharmacological activities (Vitale *et al.*, 2012). Kim *et al.* (2021) also reported that isoflavones such as genistein and daidzein exert protective effects against oxidative stress, inflammation, and related disorders.

In the present study, which aimed to evaluate the role of soybean seed methanolic extract in wound healing—a process characterized by inflammation and immune activation—we observed that the rate of healing in the treated group was slower than that of the control group, based on gross wound appearance and percentage wound contraction. Previous studies suggest that the phytoestrogenic activity of flavonoids, which are particularly abundant in soybeans, is modulated by sex and endogenous hormonal levels (Alwerdt *et al.*, 2019), potentially reducing the healing rate. Flavonoids may function either as weak estrogen agonists or antagonists depending on circulating estrogen levels and estrogen receptor expression (Kuiper *et al.*, 1998), potentially contributing to delayed wound healing in this context.

This dual behavior may explain the delayed healing observed in male albino rats treated with the soybean extract, where flavonoids could have exerted antagonistic effects. Estrogen receptors are distributed across various skin cell types—keratinocytes, sebaceous glands, hair follicles, dermal fibroblasts, and melanocytes—and are more prevalent in females than in males (Sagili *et al.*, 2021). As such, the pharmacological responsiveness to isoflavones may be attenuated in males.

Although Zhao *et al.* (2018) reported that dietary supplementation with low molecular weight soybean protein-derived peptides significantly ameliorated burn injury in rats by modulating systemic inflammatory markers (e.g., IFN-γ, MCP-1, and MCP-3) and suppressing the expression of muscle atrophy- and autophagy-related proteins (MuRF1, Atrogin-1, LC3, and Beclin-1), our findings—focused on the topical application of whole soybean extract—contradict these results in terms of macroscopic wound healing outcomes.

Similarly, in a related study by the same authors, Zhao *et al.* (2019) showed that dietary soybean peptides reduced inflammation, accelerated wound closure, and promoted tissue regeneration in a rat burn injury model. They reported decreased NF-κB signaling, reduced neutrophil and macrophage infiltration, and enhanced angiogenesis via increased CD31 expression. Again, these effects contrast with our findings, suggesting that the form of administration (dietary peptides vs. topical whole extract) and the wound model may significantly influence outcomes.

Draganidis *et al.* (2016) also noted that isoflavone-rich soy protein exerts antioxidant and anti-inflammatory effects, including modulation of NF-κB signaling, and reduces chronic inflammation and oxidative stress. While these findings support the potential of soybean-derived bioactives in tissue repair, our gross assessment revealed that topical treatment with the soybean extract did not outperform the control group treated with sterile water.

The neutrophil-to-lymphocyte ratio (NLR) is a recognized biomarker of inflammation and wound healing (Johan *et al.*, 2024). In this study, the inflammatory response was assessed during the proliferative phase, when inflammation is typically resolving. No significant difference in NLR was observed between groups. Neutrophils play an early role in host defense and debris clearance, while lymphocytes contribute to angiogenesis and extracellular matrix remodeling (Baht *et al.*, 2018; Johan *et al.*, 2024). Despite delayed healing in the treated group, the comparable NLR values may reflect the anti-inflammatory effects of flavonoids (Dower *et al.*, 2015; Esposito *et al.*, 2014).

This aligns with the findings of Zhang *et al.* (2018), who reported the immunomodulatory and anti-inflammatory effects of soybean protein (SBP) and soybean oligopeptides (SBO) in a mouse model of *Staphylococcus aureus*-induced epidermal trauma with negative nitrogen balance. They found that both SBP and SBO increased serum immunoglobulins (IgM, IgG, IgA) and downregulated inflammatory chemokines such as macrophage inflammatory protein-2 (MIP-2) and RANTES. Notably, SBO outperformed SBP in enhancing IgG levels and suppressing MIP-2, suggesting that low-molecular-weight soy peptides may provide greater immunological and healing benefits than intact proteins.

Furthermore, elevated serum and tissue levels of malondialdehyde (MDA), a key marker of oxidative stress, were observed in the treatment group, which may have contributed to delayed healing. Oxidative stress, resulting from excessive reactive oxygen species (ROS), is a critical factor in impaired wound repair (Sanchez *et al.*, 2018). Although increased serum SOD activity in the treatment group supports the antioxidant capacity of soybean isoflavones, this effect did not extend to tissue SOD levels, which were not significantly different. Previous studies emphasize that maintaining a balance between oxidants and antioxidants is essential for optimal tissue regeneration (Dunnill *et al.*, 2015).

CONCLUSION AND LIMITATION

In conclusion, the methanolic extract of soybean seeds did not enhance wound healing in male albino rats under the conditions of this study. Despite its documented anti-inflammatory properties, the extract was associated with a slower rate of wound recovery compared to the group treated with sterile water. Although it did not significantly reduce pro-inflammatory markers, the extract appeared to support antioxidant activity, suggesting that its therapeutic potential may lie more in mitigating oxidative stress than in directly modulating the inflammatory response.

A key limitation of this study was the absence of external positive controls for the quantitative phytochemical analyses. Due to resource constraints, only semi-quantitative methods were employed, which limited the precision and reproducibility of the findings. Future studies should incorporate validated positive controls to enable absolute quantification and more rigorous characterization of the extract's bioactive constituents.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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AI DECLARATION

No generative artificial intelligence tools were used in the writing, analysis, or interpretation of the data presented in this manuscript. All content is the original work of the authors.

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