

Original Article

Evaluation of the wound-healing activity of triterpenoid saponins isolated from *Momordica cymbalaria* in streptozotocin-induced diabetic rats

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ABSTRACT

Objectives: Saponins have shown promising wound-healing activity, and *Momordica cymbalaria* (Family: *Cucurbitaceae*) has been reported for its antidiabetic properties. This study aims to isolate saponins from *M. cymbalaria* and evaluate their antidiabetic and wound-healing activity in diabetic rats. The study assesses the efficacy of a saponin-based ointment (triterpenoid saponins of *M. cymbalaria* [TSMC]) on wound healing in diabetic conditions using excision and incision wound models in Wistar rats.

Materials and Methods: The roots of *M. cymbalaria* were used for saponin extraction, and the TSMC extract underwent an acute dermal toxicity study (Organisation for Economic Co-operation and Development 434). Diabetes was induced in Wistar rats using streptozotocin (STZ) (45 mg/kg, i.v.), and the animals were divided into five groups: Normal wound control (G1), diabetic wound control (G2), diabetic rats treated with 5% povidone ointment (G3), 0.5% TSMC ointment (G4) and 0.75% TSMC ointment (G5). Excision and incision models were used to assess wound-healing parameters, including epithelialisation period, wound contraction, skin tensile strength, antioxidant enzyme levels, hydroxyproline content and histopathological changes.

Results: The excision wound model showed significantly lower wound contraction and granulation tissue weight in G2 rats compared to G1 ($P < 0.001$). Treatment with TSMC (G3, G4 and G5) significantly improved wound contraction, hydroxyproline content and catalase and superoxide dismutase levels, while reducing lipid peroxidation levels ($P < 0.001$). The incision wound model revealed decreased tensile strength in G2 ($P < 0.001$), whereas G3, G4 and G5 exhibited significantly higher tensile strength ($P < 0.01$). These results suggest that TSMC enhances wound healing by promoting antioxidant activity, collagen synthesis and tissue regeneration in STZ-induced diabetic rats.

Conclusion: The study confirms that TSMC ointment, containing saponins from *M. cymbalaria*, exhibits significant wound-healing potential in diabetic conditions. Treatment with 0.75% w/w TSMC ointment accelerated wound contraction, reduced epithelialisation time, enhanced tensile strength and improved biochemical markers of healing. Histological findings further validated enhanced collagen formation and reduced inflammatory response in the treated groups. The results suggest that TSMC could be a promising therapeutic agent for diabetic wound healing, warranting further clinical investigations.

Keywords: Antioxidant activity, *Cucurbitaceae*, Diabetic wound, Hydroxyproline, *Momordica cymbalaria*, Triterpenoid saponins

INTRODUCTION

Alternate systems of medicine, such as Ayurveda and Siddha, have gained prominence in recent times. Such systems of medicine employ herbal preparations, which are preferred due to their low toxicity in comparison to drugs of synthetic origin. Through scientific standardisation, the effectiveness of plants that have long been utilised for their diverse wound-healing qualities is investigated. One to 3% of medications in Western pharmacopoeia are available for treating skin conditions and wounds, with one-third coming from natural sources. Since ancient times, plants have been used to treat a range of dermatological and epidermal issues, especially burns, wounds and cuts.^[1]

Triterpenoid saponins of *Momordica cymbalaria* (TSMC) (*Cucurbitaceae*) are reported to have antidiabetic,^[2,3] cardioprotective,^[4] anti-ovulatory and anti-ulcer activity,^[5] anti-implantation^[6] and abortifacient activities. Antimicrobial action has also been observed for TSMC.^[7] The present study seeks to assess the wound-healing potential of TSMC saponins in diabetic rats.

The clinical concept of a wound is the breach or loss of the cellular continuity of living tissues. Daily activities can lead to damage, which requires treatment for the resulting wounds. Healing of wounds is a natural and complex restorative response to tissue injury with very orderly programmed events and is categorised into four overlapping phases: Haemostasis, inflammatory phase, proliferation and remodelling.^[8] Diabetes and its complications, such as delayed wound healing, peripheral neuropathy and nephropathy, have become a serious clinical issue in society and need to be addressed.^[9]

Worldwide, nearly 8.32 million people suffer from different wounds (it is 8.32 million).^[10] In recent years, additional studies have been conducted to further understand wound prevalence in India. For instance, a study conducted between June 2022 and December 2023 in Varanasi district screened 10,003 individuals and found an overall chronic wound prevalence of 1.89/1,000 people. The prevalence was 1.57/1,000 in urban areas and 2.64/1,000 in rural communities.^[11] According to the study, there are 15.03 wounds/1000 Indians, of which there are 4.5 chronic wounds and 10.5 acute wound cases. By 2027, the global market for wound care is expected to reach \$18.7 billion, with a 6.6% compound annual growth rate between 2020 and 2027.^[11] As a result, the demand for medications for wound healing has increased.

Diabetes mellitus, a metabolic condition resulting in increased blood sugar levels, is associated with complications such as impaired wound healing due to connective tissue abnormalities.^[12] The connective tissue's main component, collagen, is crucial for wound healing.^[13] Diabetes-related

delayed wound healing is primarily caused by a drop in skin collagen content^[14] and reduction or absence of growth factors, especially cytokines responsible for molecular and cellular signalling for normal wound healing.^[15]

The selection of *M. cymbalaria* and its saponins for this study is based on their traditional use in Ayurvedic medicine for treating inflammatory disorders and their demonstrated bioactive properties. Saponins, key phytochemicals in *M. cymbalaria*, have been reported to promote fibroblast proliferation, enhance collagen synthesis and exhibit antioxidant effects, all of which are crucial for effective wound healing. Given that diabetic wounds often suffer from impaired fibroblast function, oxidative stress and delayed collagen deposition, the antioxidant and pro-healing effects of saponins present significant therapeutic potential. Despite its known pharmacological benefits, the role of *M. cymbalaria* saponins in diabetic wound healing remains underexplored. Therefore, this study aims to bridge this gap by evaluating the efficacy of these saponins in promoting wound healing, enhancing collagen strength and reducing oxidative injury in diabetic rats, thereby providing a strong basis for their translational application in diabetic wound management.^[16]

MATERIALS AND METHODS

Chemicals and reagents

Streptozotocin (STZ), hydroxyproline, Ehrlich's reagent, glucosamine hydrochloride, acetaminophen hydrochloride, acetylacetone (Sigma Aldrich), Xylazine hydrochloride (Xylaxin), Ketamine hydrochloride i.p and Poviz-MA (Zenith Drugs Pvt., Ltd.).

Extraction and isolation of TSMC

The roots of *M. cymbalaria*, about 1 kg, were subjected to Soxhlet extraction with 3 L of methanol for 8–10 h at 64–68°C. Further, 140 g of methanolic extract was dissolved in 500 mL of hot distilled water. This was partitioned between n-butanol and water. Then, the n-butanol layer was evaporated to get a residue. This residue was dissolved by adding 200 mL of diethyl ether to obtain a precipitate. The precipitate was separated and dried the percentage of yield was calculated and subjected to phytochemical investigation.^[17]

Formulation of ointment

Ointments of 0.5% and 0.75% w/w were prepared using soft white paraffin.^[18]

Experimental animals

Wistar rats (Male) weighing between 200g and 250g were procured and housed in a cage under standard laboratory

conditions. The room temperature is maintained at $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$ with a 12 h light and dark cycle. The animals were provided with normal diets, namely, chow and water *ad libitum*, except during experimentation. The study protocols were duly approved by the Institutional Animal Ethics Committee (1564/Po/a/11/committee for the purpose of control and supervision of experiments on animals [CPCSEA]) of Karnataka College of Pharmacy, Bengaluru. Studies were performed following the CPCSEA guidelines.

Acute dermal toxicity

An acute dermal toxicity study protocol was carried out according to the Organisation for Economic Co-operation and Development guidelines no. 402. The highest dose of TSMC (10%w/w) was applied to the dorsal region of the Wistar rats.^[2]

Induction of diabetes

Diabetes induction was done using STZ (45 mg/kg, i.e. single dose) in ice-cold 0.1 M citrate buffer, pH 4.0. Rats having fasting blood glucose levels >250 mg/dL will be classified as diabetic and added to the study. Blood glucose levels will be checked using a glucometer after 72 h. It has been reported that the STZ-induced diabetes model in rodents will decrease cutaneous wound strength.^[19]

Experimental grouping of animals

The excision and incision wound model was used to determine the potential of TSMC in diabetic rats. The animals were split up into the following groups (G) in five groups ($n = 6$). In this work, two experimental models were used. The treatment regimen is as follows,

- G₁: Normal wound control treated with simple base ointment
- G₂: Diabetic wound control treated with simple base ointment
- G₃: Treated with Povidone ointment as a standard group
- G₄: Treated with TSMC ointment 0.5%w/w (100 mg ointment per rat)
- G₅: Treated with TSMC ointment 0.75%w/w (100 mg ointment per rat).

Excision wound model

In this experimental model, ketamine (0.5mL/kg, i.p.) is used as an anaesthetic agent, and normal control group rats were anaesthetised on the 7th day. In each rat, the right side was shaved, and 4 cm² excision wounds were made. Animals received different treatments as per the regimen described above. Graph paper was used to mark the wound area on the 2nd day to measure the wound contraction. By removing the

granulation tissue from the wound site, several parameters are estimated, including hydroxyproline^[20] and antioxidant activity such lipid peroxidation (LPO), catalase (CAT) and superoxide dismutase (SOD).^[21] Analysis of glucose, cholesterol and triglycerides was done by collecting blood through the retro-orbital vein after complete recovery of the wound. Further, the alteration in wound size was measured using the rate of wound retrenchment through a translucent sheet having a millimetre scale. Furthermore, the period of epithelialisation was measured. One of the biochemical estimations, like the hydroxyproline content of excision wounds, was envisaged with reference to the previously reported method.^[22]

Incision wound model

In this experimental study, rats' dorsal part was shaved and they were anaesthetised with ketamine (0.5 mL/kg b. w. i.p.). Incision wounds were made with a sterile scalpel by making a cut about 6 cm in length and 2 mm in depth on the skin. The parted skin was stitched at 0.5 cm intervals using black silk. Surgical thread (no. 000) and a curved needle (no. 9) were used for stitching. All the groups received their respective treatment for 10 days. The wounding day was counted as day 0. Sutures were removed on the 8th post-wounding day, and tensile strength was measured with a tensiometer on the 10th day.^[10]

Statistical analysis

The data were represented as mean + SEM and analysed by one-way analysis of variance and Dunnett's *t*-test using GraphPad Prism of Biodata Corporation-version 5. The data were considered statistically significant at $P < 0.05$.

RESULTS

According to the present study's isolation results, TSMC's pure saponin yield was 0.00855%. Phytochemical analysis of the isolated saponin of TSMC showed positive results for saponins and phytosterols [Figure 1].

Acute dermal toxicity study results revealed that on observation, there was no evidence of toxicity or death at a dose of 2000 mg/kg body weight. As a result, 0.5% and 0.75%w/w of TSMC were taken for the study.

The wound contraction and epithelialisation duration (days) of the excision wound model demonstrated that G₂ rats had significantly lower wound contraction percentages on days 4, 8, 12 and 16 compared to G₁ rats ($P < 0.001$) [Figure 2]. On the other hand, on days 4, 8, 12 and 16, the percentage of wound contraction increased significantly ($P < 0.001$) in G₃, G₄ and G₅ rats. Compared to G₁ animals, the weight of the moist granulation tissue

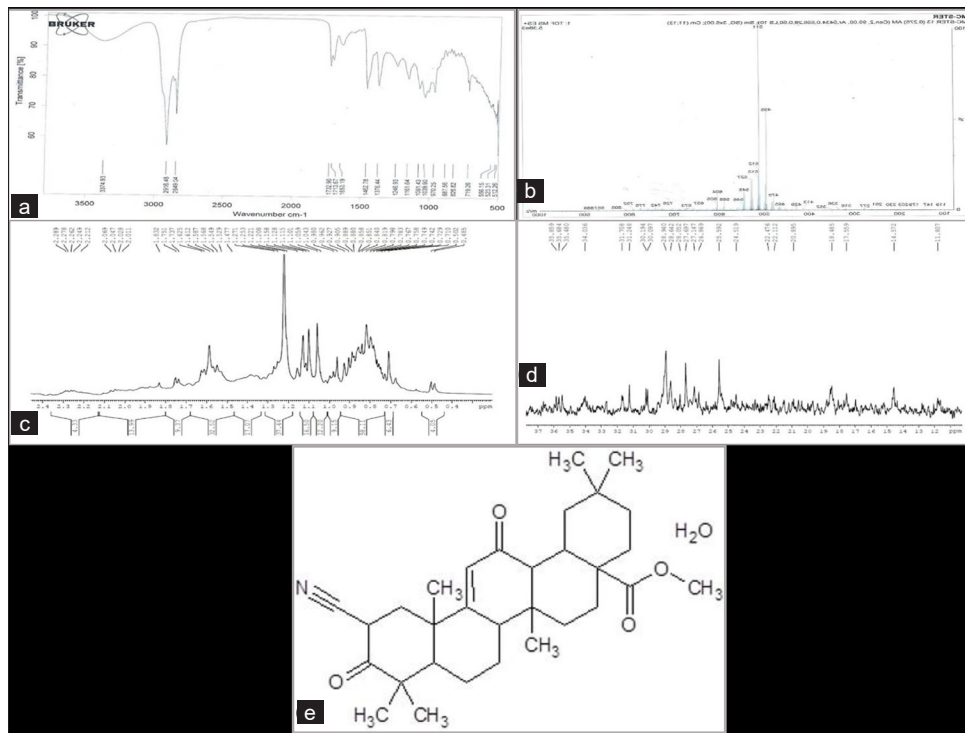


Figure 1: Chromatogram showing (a) FTIR analysis of TSMC, (b) mass spectra analysis of TSMC, (c) ¹H NMR spectra analysis of TSMC, (d) ¹³C NMR spectra analysis of TSMC, and (e) Structure of TSMC.

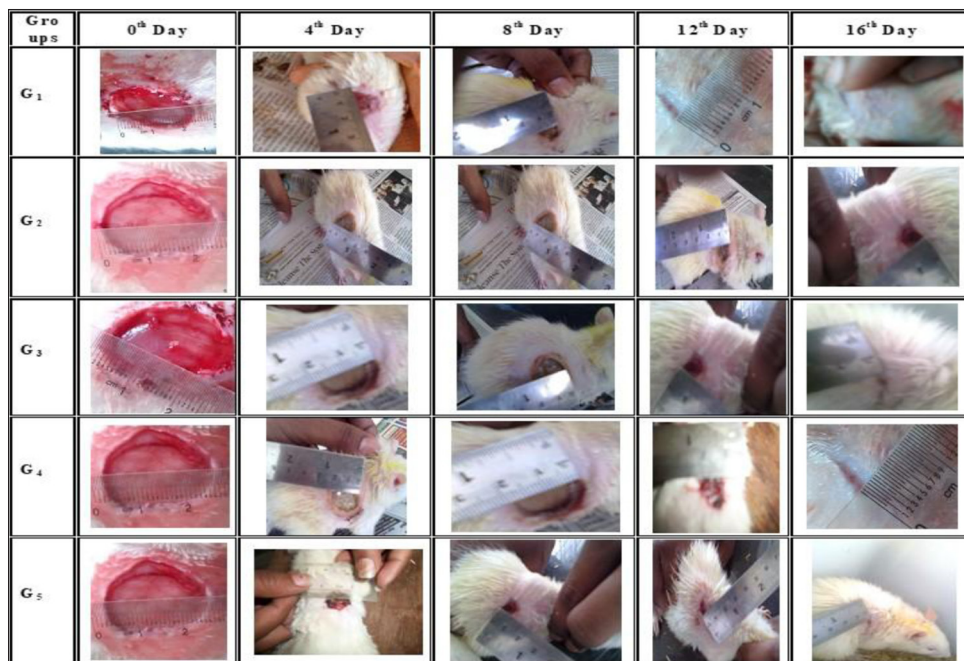


Figure 2: Representative photographs showing wound on day 0, 4th, 8th, and 16th postoperative day.

decreased significantly ($P < 0.001$) in the G2 rats. When compared to G2 rats, wet granulation tissue weight

increased significantly ($P < 0.001$) in the G3, G4 and G5 rats [Figure 3 and Table 1].

The hydroxyproline content in G₂ rats was significantly lower ($P < 0.001$) when compared with G₁ rats. The hydroxyproline content of the G₃, G₄ and G₅ rats increased significantly ($P < 0.001$) when compared to the G₂ animals [Table 2 and Figure 4].

Evaluation of the antioxidant property of TSMC in the diabetic rat groups revealed that CAT and SOD levels of G₂ rats in granulation tissue were significantly decreased ($P < 0.001$) when compared with the G₁ rats. The G₃, G₄ and G₅ rats exhibited a significant ($P < 0.01$) increase in CAT and SOD levels in the granulation tissue when compared with G₂ rats ($P < 0.001$) [Table 3].

In this research, the antioxidant effect on LPO was studied. Comparing G₂ rats to G₁ rats revealed a substantial ($P < 0.001$) rise in LPO levels in the granulation tissue. However, as compared to G₂, diabetic wounds treated with G₃, G₄ and G₅ exhibit significantly reduced LPO levels ($P < 0.001$) in the granulation tissue [Figure 4].

Results of the incision wound model revealed that G₂ rats showed significantly decreased ($P < 0.001$) tensile strength

when compared with G₁ rats. While diabetic wounds treated with G₃, G₄ and G₅ recorded high tensile strength ($P < 0.001$) ($P < 0.01$), respectively.

Histopathology study of diabetic rats in the various groups in the present study showed that G₁ rats showed no haemorrhage, complete epithelisation, as well as diffuse dermal fibrous connective tissue proliferation, which was observed. G₂ rats' endothelial proliferation and the placement of newly formed blood vessels perpendicular to the fibrous connective tissue revealed insufficient epithelisation, a thick keratin layer, loosely spaced collagen fibres and the absence of dermal structures. G₃, G₄ and G₅ revealed complete epithelisation, fewer inflammatory cells, dispersed cutaneous fibrous connective tissue growth as well as tiny haemorrhages and endothelial proliferation, with the organisation of newly generated blood vessels perpendicular to fibrous connective tissue [Figure 5].

DISCUSSION

The present study's findings indicate that in experimental diabetic rats, the TSMC enhances wound healing. This

Table 1: Effect of isolated Triterpenoid saponins of *Momordica cymbalaria* (TSMC) on Wound contraction and epithelization period in excision wound diabetic rat model.

Groups (n=6)	% Wound contraction				Epithelization period (days)
	4 th Day	8 th Day	12 th Day	16 th Day	
Group 1 Normal wound control	20.43±0.15	46.24±0.11	84.30±0.66	99.01±0.18	14.17±0.16
Group 2 Diabetic wound control	8.413±0.05 ^{***a}	31.88±0.19 ^{***a}	52.45±0.32 ^{***a}	67.88±0.12 ^{***a}	26.17±0.16 ^{***a}
Group 3 Diabetic wound+Povidone	18.00±2.00 ^{***b}	47.00±1.00 ^{***b}	72.67±0.843 ^{***b}	95.77±0.09 ^{***b}	16.17±0.16 ^{***b}
Group 4 Diabetic wound+0.5%w/w TSMC	13.20±0.45 ^{***b}	35.90±0.46 ^{***b}	64.65±0.143 ^{b***}	85.86±0.11 ^{***b}	17.83±0.16 ^{***b}
Group 5 Diabetic wound+0.75%w/w TSMC	18.33±0.21 ^{***b}	35.67±0.91 ^{***b}	71.87±0.169 ^{***b}	92.67±1.22 ^{***b}	17.83±0.16 ^{***b}

Values are expressed as mean±SEM. Data were analyzed by one-way ANOVA followed by Dunnett's test. The number of animals in each group is n=6. ^acomparison was made with the normal wound group. ^bComparison made with diabetic wound control group. ^{***} $P < 0.001$

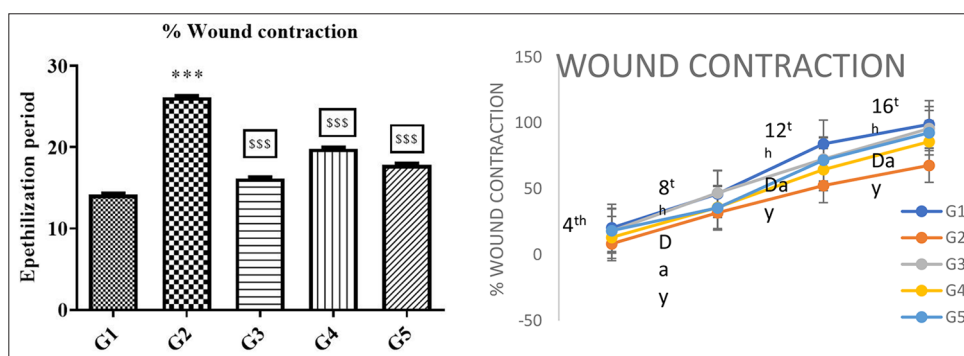


Figure 3: Effect of TSMC on % wound contraction. The data were considered statistically significant at $P < 0.05$. Data were analyzed by one-way ANOVA followed by Dunnett's test. Number of animals in each group n=6, compared with G₁, ^{***} $P < 0.001$. Compared with G₂, ^{***} $P < 0.001$.

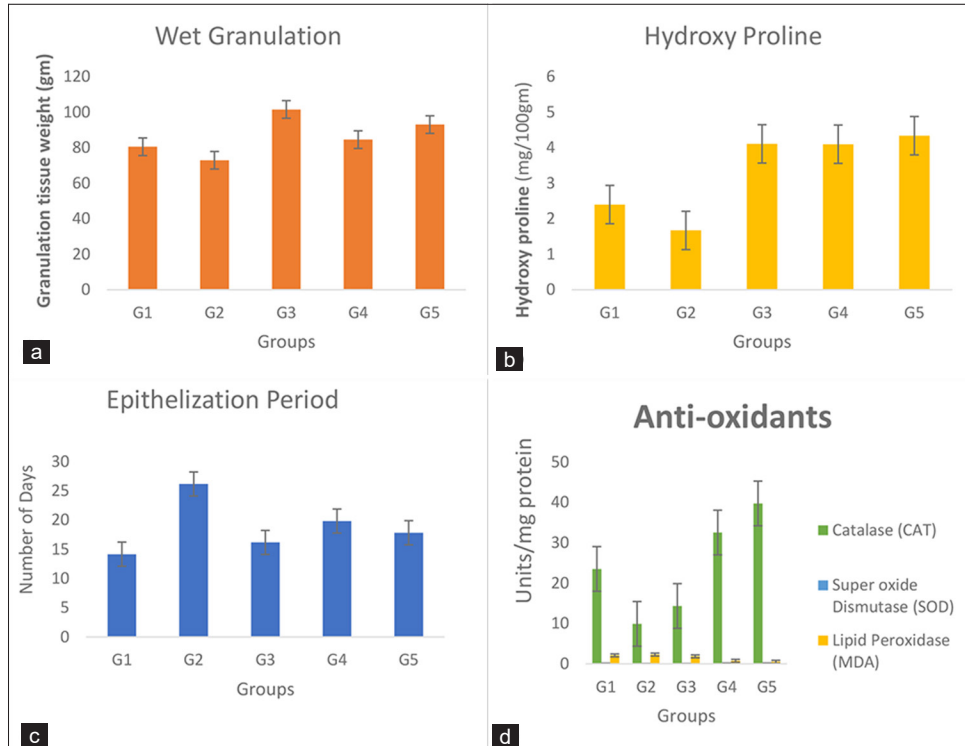


Figure 4: Effect of TSMC on (a) wet granulation weight, (b) hydroxyproline content, (c) epithelialization period. The data were considered statistically significant at $P < 0.05$. Data were analyzed by one-way ANOVA followed by Dunnett's test. Number of animals in each group $n = 6$. (d) Effect of TSMC on catalase, SOD & lipid peroxidase (MDA). The data were considered statistically significant at $P < 0.05$. Data were analyzed by one-way ANOVA followed by Dunnett's test. Number of animals in each group $n = 6$.

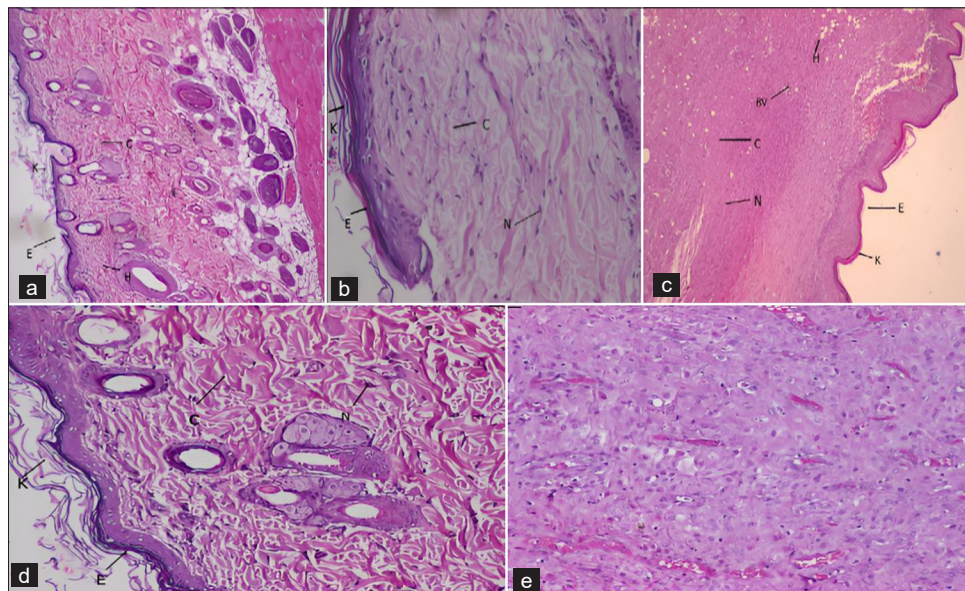


Figure 5: Histological study of different groups. (a) G1-Normal wound control, (b) G2- Diabetic wound Control Group, (c) G3: - Povidone ointment treated, (d) G4-0.5%w/w TSMC, (e) G5-0.75%w/w TSMC.

was shown by a substantial rise in the decreased period of epithelisation, hydroxyproline content, anti-oxidant activity, LPO and rate of wound contraction. Therefore, TSMC is found to have wound-healing activity and is confirmed by excision and incision models in diabetic wound rats.

Wound healing is a sequential process that consists of several interrelated stages, such as haemostasis, inflammation, proliferative and remodelling and maturation. Wound healing is a natural process governed by the body's inherent resistance mechanism. The body's own immune system can repair formed wounds by cellular mechanisms.

Table 2: Effect of isolated Triterpenoid saponins of *Momordica cymbalaria* (TSMC) on Wet- granulation tissue weight and Hydroxyproline Content in excision wound diabetic rat model.

Groups (N=6)	Wet granulation tissue weight (mg)	Hydroxy proline (mg/100 g of tissue)
Group 1 Normal wound Control (Untreated)	80.58±1.11	2.408±0.05
Group 2 Diabetic wound control (Untreated)	72.97±1.45***a	1.670±0.07***a
Group 3 Diabetic wound+Povidone cream (5% w/w)	101.6±1.15***b	4.115±0.064***b
Group 4 Diabetic wound+0.5%w/w TSMC	84.59±0.88***b	4.109±0.030***b
Group 5 Diabetic wound+0.75%w/w TSMC	93.05±0.69***b	4.346±0.07***b

Values are expressed as mean±SEM. Data were analyzed by one-way ANOVA followed by Dunnett's test. The number of animals in each group was n=6. ^acomparison was made with the normal wound group. ^bComparison made with diabetic wound control group. ***p<0.001

Wounds heal at an optimal rate in most cases. Diabetes, ischemia, and immunological diseases may all cause delayed wound healing. Diabetes-related impaired wound healing is a significant comorbidity. Diabetes is a chronic hyperglycaemic condition that inhibits cell proliferation,^[5] resulting in decreased collagen synthesis, prolonged inflammation and inadequate macrophage function. In addition, diabetic wounds are susceptible to infection due to impaired granulocyte function and chemotaxis. This research is mainly focused on delayed wound healing in experimental diabetic rats.

To investigate the healing potentials of TSMC at various stages of wound healing, two distinct models (excision and incision) are used.

Wound contraction is an intricate process where narrowing or closing occurs with well-orchestrated interaction of cells, extracellular matrix and cytokines. As the movement of fibroblasts takes place, it facilitates matrix formation and collagen in the wound area.^[23] Diabetic wound control rats showed a significantly lower proportion of wound contraction than the normal wound control group; this could be due to decreased proliferation. Reduced expression of growth factors such as platelet-derived growth factor, insulin-like growth factor, transforming growth factor (TGF) and nerve growth factor may be the cause of this protracted wound healing.^[24]

Diabetic circumstances cause the amount of collagen in the skin to decrease due to impaired production and degradation of newly created collagen.^[25] When compared to diabetic wound control groups, it was found from the current investigation that diabetic wounds treated with conventional ointment and test doses of TSMC displayed a significant increase in the percentage of wound contraction. This might be caused by an increase in fibroblast cell proliferation and myofibroblast differentiation.^[26] Myofibroblasts, the primary component of the wound contraction machinery located at the wound margin, have increased in quantity, which contributes

Table 3: Effect of Triterpenoid saponins of *Momordica cymbalaria* (TSMC) in an excision wound diabetic rat model.

Groups (N=6)	Catalase (CAT)	Super oxide dismutase (SOD)	Lipid peroxidase (MDA)
Group 1 Normal wound control	23.53±1.74	0.14±0.010	2.146±0.043
Group 2 Diabetic wound control	9.913±0.26***a	0.065±0.002***a	2.372±0.06***a
Group 3 Diabetic wound+Povidone Cream (5% w/w)	14.35±1.080***b	0.069±0.003***b	1.863±0.056***b
Group 4	32.54±3.00***b	0.1650±0.009***b	0.7628±0.039***b

Values are expressed as mean±SEM. N=6. ***p<0.001 When compared to normal control; ***p<0.001 When compared to diabetic control

to faster wound healing. These are in charge of covering debris, which promotes quicker wound healing.^[27]

Formulations that are applied topically, like ointments, are efficient in hastening wound contraction because they are more readily available at the wound site. In the present study, it was discovered that rats treated with TSMC had a higher rate of wound contraction.

The epithelialisation period of the wound was expressed as the number of days taken for complete epithelialisation. In this phase, the process of drifting the keratinocytes from the lower skin occurs, followed by dividing.^[28] When compared to a control group of rats with normal wounds, the diabetic wound control rats in the present study displayed a much longer epithelialisation duration. In comparison to the control group of diabetic wounds, diabetic wounds treated with conventional ointment and test doses of TSMC displayed a considerable shortening of the epithelialisation period. This might be brought on by cell migration and growth.^[5] In addition, wet granulation tissue weight in diabetic wounds treated with standard and test doses of TSMC in rats increased significantly compared to the diabetic wound control group. The presence of more protein is indicated by an increase in the granulation tissue's weight.^[10]

In granulation tissue, extracellular protein like collagen predominantly helps in wound healing. After an injury, a rapid increase in the synthesis of collagen will strengthen the integrity of the tissue matrix and play an important role in haemostasis as well as in epithelialisation.^[10] The amino acid hydroxyproline makes up the collagen fibres in granulation tissue, and measuring it can help clinicians better understand how quickly the healing of a wound's connective tissue is progressing.^[25]

Compared to the normal wound control group in the current investigation, diabetic wound control rats revealed a significant drop in the hydroxyproline content ($P < 0.001$), which may be related to lower collagen levels in diabetic settings.^[21] The diabetic wound-treated group (G) with standard and test doses of TSMC showed a significant increase in the hydroxyproline content compared to the diabetic wound control group. These increased levels of collagen in the granulation tissue would have contributed to fast healing and strengthened the repaired tissue.^[25] A higher concentration of hydroxyproline suggests that wounds heal more quickly.

Histopathological images illustrated that the normal wound control group showed no haemorrhage, diffuse dermal fibrous connective tissue proliferation well as complete epithelialisation. In G1, endothelial growth was seen along with the placement of newly created blood vessels parallel to fibrous connective tissue [Figure 5a]. In contrast, rats with diabetic wound control showed loosely distributed collagen fibres, a dense keratin layer, partial epithelialisation

and no dermal structures. This might be caused by diabetes [Figure 5b]. However, the histological analysis revealed that diabetic wounds treated with standard [Figure 5c] and test doses of TSMC [Figures 5d and e] displayed complete epithelialisation, a decrease in the number of inflammation cells, diffused dermal fibrous connective tissue proliferation, small haemorrhages and endothelial proliferation with the arrangement of newly formed blood vessels perpendicular to fibrous connective tissue. This might be because the TSMC's improved chemotactic impact, which might have drawn inflammatory cells to the wound site, caused a rise in cellular infiltration.^[10]

Mitogenic activity of the plant may contribute to the increase in cellular proliferation, and this, in turn, might result in the healing process. The extract had a favourable impact on cellular proliferation, the development of granular tissue and the epithelialisation of dermal and epidermal regeneration, according to earlier investigations on the treatment with the extract. This research is in concurrence with the study by Gupta *et al.*^[28]

Cell proliferation, inflammation and contraction of collagen lattice formation are important steps in wound healing. At the wound site, conventional symptoms such as pain, reddening and oedema were triggered by the release of reactive oxygen species, prostaglandins, eicosanoids and leukotrienes. At the same time, free radicals' presence may hamper wound healing by damaging the cells surrounding wounds.^[10]

When compared to the normal wound control group in the current investigation, the diabetic wound control rats revealed a substantial decrease in CAT and SOD levels in the granulation tissue, which may be related to the extract's free radical scavenging action. Advanced glycation end products, which limit vascularisation and cause the imperfect creation and disruption of blood vessel gap junctions, are promoted by elevated blood sugar levels by increasing oxygen-free radical production. Increased levels of blood glucose also inhibit haemangiogenesis by promoting the generation of tumour necrosis factor (TNF)-alpha (TNF-s). Several factors are involved in decreasing microvascular circulation and, thereby, cause impaired wound healing in diabetes.^[19] When compared to the diabetic wound control group, diabetic wounds treated with conventional and test doses of TSMC showed significantly higher CAT and SOD levels in the granulation tissue. This shows that the scavenging action was repairing the tissue damage.^[29]

Compared to the normal wound control group, diabetic wound control rats had significantly higher LPO levels in the granulation tissue. This could be caused by a rise in free oxidative radicals, a fall in antioxidant defence systems, or both. Both people and rats have had similar experiences. When compared to the diabetic wound control group, diabetic wounds treated with standard and

test doses of TSMC demonstrated significantly lower LPO levels (*P*-values) in the granulation tissue, which may be attributable to a decline in lipid oxidation, a reduction in the production of free oxidative radicals or an increase in antioxidant defines mechanisms.

A healing wound's tensile strength can be empirically determined by calculating the amount of force necessary to rupture it.^[10] The amount of freshly produced collagen deposited at the wound site directly correlates with tensile strength. The marked upsurge in the breaking strength specifies improved wound healing. The tensile strength of the skin is confirmed by the constant accumulation of collagen, the proliferation of fibroblasts and a marked reduction in leukocyte invasion and oedema. This signifies ruptured surface is firmly knitted by collagen.^[30]

In this study, the tensile strength of the diabetic wound control rats was significantly lower than that of the normal wound control group. The difference in tensile strength between diabetic wounds treated with conventional and test dosages of TSMC and the control group for diabetic wounds may be attributable to the deposition of firmly knit collagens at the ruptured surface of the wound. Supporting myofibroblast growth and angiogenesis is crucial for maintaining wound tensile strength. The expanded ductile quality uncovers that the disturbed surfaces are immovably woven by collagen. Similar studies have been recorded. TGF- β and vascular endothelial growth factor (VEGF) play crucial roles in angiogenesis and vascular development through complex signalling mechanisms. TGF- β acts as both a promoter and inhibitor of angiogenesis depending on the cellular context. It regulates VEGF expression in osteoblasts and osteoblast-like cells, with TGF- β 1 inducing a dose-dependent increase in VEGF protein expression.^[31] The enhanced wound contraction and tensile strength may be mediated through the upregulation of key growth factors such as TGF- β and VEGF, which play crucial roles in fibroblast proliferation, collagen synthesis and angiogenesis. The increased hydroxyproline levels indicate enhanced collagen deposition, supporting improved extracellular matrix remodelling. Furthermore, the antioxidant activity of saponins may contribute to reduced oxidative stress, promoting faster tissue regeneration.^[32]

Summary

The present study indicated that the saponins of *M. cymbalaria* promote wound-healing activity in diabetic rats. Wound-healing activity was determined through both incision and excision wound models. In diabetic rats, the extract showed a significant increase in the rate of wound contraction. An increase in wound contraction may be due to an enhanced activity of fibroblasts in regenerated wound tissue. The result of the hydroxyproline level shows the increased collagen strength in the regenerated wound tissue.

Determination of antioxidant activity and LPO shows a decrease in oxidative injury, which may be due to increased oxygen-free radical scavenging. This may be due to increased oxygen-free radical scavenging of saponin. An increase in tensile strength may be due to the deposition of newly synthesised collagens at the wound site.

CONCLUSION

The present study demonstrated that saponins from *M. cymbalaria* significantly enhance wound healing in diabetic rats by promoting wound contraction, fibroblast activity, collagen deposition and tensile strength in both incision and excision wound models. Increased hydroxyproline levels confirmed enhanced collagen synthesis, while antioxidant and LPO assays indicated reduced oxidative stress, likely due to the extract's free radical scavenging potential. These findings suggest strong translational potential for *M. cymbalaria* saponins as a phytotherapeutic agent for diabetic wound care, with possible applications in topical hydrogel formulations, bioactive wound dressings or injectable therapies.

To advance toward clinical use, structured clinical trials are necessary, starting with preclinical toxicology and pharmacokinetics studies, followed by a phase I safety and tolerability trial in healthy volunteers. Phase II randomised controlled trials in diabetic patients with chronic ulcers would assess efficacy, wound contraction rate, collagen synthesis and oxidative stress markers. A large-scale phase III trial would compare its effectiveness against standard wound care treatments, evaluating complete wound closure and healing time. Finally, Phase IV post-market surveillance would ensure long-term safety and clinical effectiveness. With its wound-healing potential, antioxidant properties and fibroblast-stimulating effects, *M. cymbalaria* saponins could offer a cost-effective, plant-based therapeutic alternative for managing diabetic wounds, improving healing outcomes and reducing complications.

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