

Wound healing property of topical application of ethanolic extract of *Michelia champaca* flowers in diabetic rats

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ABSTRACT

Background: The plant *Michelia champaca* (MC) is widely used in the treatment of inflammation, constipation, dysmenorrhea, ulcers, wounds, fever and cough. The objective of the study was to evaluate the wound healing property of topical application of ethanolic extract of MC flowers in streptozotocin induced diabetes in rats.

Materials and Methods: Wound healing activity was assessed by incision and excision wound models. Five groups of 10 rats each were used for each of incision and excision wound model. Group I rats, non-diabetic control and group II rats diabetic control, were anointed topically with ointment base. The diabetic rats in test groups III, IV and V were anointed with topical MC extract ointment of 2.5%, 5% and 10% respectively. Parameters observed were breaking strength of incision wound and wound contraction, epithelialization, hydroxyproline content of excision wound respectively. Results were analyzed by one-way analysis of variance (ANOVA), followed by Tukey's post hoc test.

Results: Breaking strength, rate of wound contraction and hydroxyproline content were significantly increased and period of epithelialization was significantly reduced in Group IV and V rats respectively.

Conclusion: The topical application of ethanolic extract of *Michelia champaca* promotes wound healing in diabetic rats. Hence further study in humans is suggested.

Key words: incision wound, excision wound, hydroxyproline and diabetic rats.

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INTRODUCTION

Wound healing is a complex phenomenon that results in the restoration of anatomic continuity and function. Wound healing involves different phases including inflammation, granulation, fibrogenesis, neo-vascularisation, wound contraction and epithelialization.^[1]

Effective management of the wound requires understanding of the normal repair process and selection of appropriate intervention to optimize process of healing. Diabetes, even in its early stages can impair the normal course of wound healing, however the underlying

mechanisms of defective wound repair in diabetes are not completely understood, but delayed collagen synthesis and accelerated degradation of newly synthesized collagen, impaired epithelialization and reduced angiogenesis have been described during proliferative phase of healing process.^[2]

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The plant *Michelia champaca* (MC) belonging to family Magnoliaceae, is widely used in both Ayurveda and Siddha medicine. Root and bark are used as purgative and in the treatment of inflammation, constipation and dysmenorrhea. The stem bark has astringent and febrifuge properties.^[3] The flower buds of MC are commonly used by many traditional healers in most of herbal preparations for diabetes.^[4] Furthermore, the extracts of flower and flower buds have shown anti-inflammatory,^[5] antipyretic^[6] and wound healing properties.^[7]

A survey of the literature revealed that wound healing activity of MC in diabetics has not been documented. Hence, this study was undertaken to evaluate the wound healing activity of MC in diabetic rats.

MATERIALS AND METHODS

Animals

Healthy, adult albino rats of Wistar strain of either sex, bred locally in the animal house of Kasturba Medical College, Manipal, weighing between 150 to 200 g were used. They were housed under controlled conditions of temperature ($23\pm 2^\circ\text{C}$), humidity ($50\pm 5\%$) and 10-14 hours of light and dark cycles^[8] The animals were housed individually in polypropylene cages with sterile paddy husk bedding. Food pellets and water were provided *ad libitum*. The study was carried out after obtaining clearance from the Institutional Animal Ethics Committee, Manipal. (IAEC/KMC/44/2010-2011)

Ethanollic extract of *M. champaca*

The flowers of *M. champaca* (MC) were procured from a local florist shop and authenticated by the Professor of botany, Mahatma Gandhi Memorial College, Udupi. A voucher specimen was preserved at the depart-

ment of pharmacology, Kasturba Medical College, Manipal, India. Flowers were shade dried and were finely powdered. The powder was loaded into Soxhlet extractor in batches of 200 g each and was subjected to extraction for 30-40 hours with 95% ethanol.^[9] After extraction, the solvent was distilled off and concentrated on a water bath at a temperature below 50°C to syrup consistency. Then it was dried and stored in a dessicator. The yield was about 10%.

Induction of diabetes

Rats were fasted overnight and their fasting blood glucose was measured. Single dose of Streptozotocin solution (STZ, 30mg kg^{-1}) in sodium citrate buffer, pH 4.5, freshly prepared was injected intraperitoneally and 10% glucose water was supplied to avoid sudden hypoglycemia post-injection.^[10] Blood glucose measurement was performed 7 days after STZ injection.^[11] Blood was drawn from the tail vein and glucose level was determined using a glucometer. Rats with blood glucose levels $>250\text{ mg/dL}$ were considered as diabetic.^[12] Rats in nondiabetic group were injected with a single dose of saline, intraperitoneally.

Study design

A total of 100 animals were used. They were divided randomly into 5 groups for each of incision ($n=6$) and excision wound ($n=14$) models. In each of the model the dose and method of drug administration was as follows;

Group I was non-diabetic control animals, which received ointment base topically. Group II was the diabetic control animals, received ointment base topically. Group III, IV and V were diabetic animals, which received extract topically at different doses such as 2.5%, 5% and 10%. The incision wound and excision wounds were created on 7th day after induction of diabetes.^[12]

Incision wound model

The animals were anaesthetized by injecting Ketamine, 80 mg/kg, intraperitoneally. The back of the rats were shaved. Two 6 cm long paravertebral straight incision were made, 1 cm lateral to the vertebral column on either side through the entire thickness of skin. Wounds were closed with intermittent sutures, 1 cm apart, with black silk thread and curved needle.^[13] Animals were treated once daily with the drugs from day 0 (day of wounding) to day 9; sutures were removed on day 7. On day 10, breaking strength of the wound was measured by applying tearing force in the form of continuous water flow technique of Lee.^[14]

Excision wound model

Under ketamine anesthesia, the back of the rats were shaved. A round seal of 2.5 cm diameter was impressed on the dorsal interscapular region, 5 cm away from the ears of the rat. Full thickness skin from the demarcated area was excised to get a wound approximately measuring 500 mm².^[15] Animals received the drugs from day of wounding to 21st postoperative day. Wound contraction rate was monitored in six animals from each group on every alternate day starting from day 0 by planimetric measurement. The wound tracings were then transferred to a 1 mm² graph paper, to determine the wound area. Wound contraction was calculated as percentage of the original wound size.^[16] Epithelialization period was monitored by noting the number of days required for eschar to fall away, leaving no raw wound behind.

On 10th postoperative day, tissue was collected from the wound using a punch biopsy needle (5 mm), for histopathological analysis.^[17] The animals from which punch biopsy was taken were not included for the assessment of wound contraction and period of epithelialization.

On 10th postoperative day, granulation tissue was collected. The granulation tissue was dried in an oven for 24 hours and the dry weight was noted. Acid hydrolysate of the dry tissue was used for the determination of hydroxyproline content.^[18]

Statistical analysis: The results were analyzed for statistical significance using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test, using SPSS computer software (version 15.0). A *p*-value < 0.05 was considered as statistically significant.

RESULTS

Incision wound model

In this wound model, the breaking strength of ten day old incision wound was measured. The mean breaking strength of wound in nondiabetic control group was 259.28 ± 7.54 g and in diabetic control group it was 234.56 ± 5.49 g. Although the mean breaking strength in group treated with 2.5% MC extract was increased to 276.22 ± 9.42 g, it was not significant. The animals that received 5% and 10% MC extract demonstrated a mean breaking strength of 303.72 ± 9.7 g and 319 ± 9.23 g, when compared with the diabetic control group. This has significant difference with a *p*-value < 0.05 compared to diabetic control and nondiabetic control groups (Table 1).

Excision wound model

The percentage of wound contraction measured on day 4, 8, 12 and 16 in nondiabetic control group was 19.32 ± 2.43, 44.82 ± 1.76, 62.71 ± 3.53 and 82.46 ± 2.43, respectively. In diabetic control group, it was significantly (*p* < 0.05) reduced to 32.06 ± 2.29, 46.2 ± 1.92 and 67.22 ± 5.41 on day 8, 12 and 16 respectively; but there was no significant decrease in wound contraction on day 4 (Table 2).

Table 1: Effect of topical *Michelia Champaca* flower extract on breaking strength in incision wound

| Group, treatment | Breaking strength (g) |
|---|-----------------------|
| Group 1, Ointment base (Non-diabetic control) | 259.28 ± 7.54 |
| Group 2, Ointment base (Diabetic control) | 234.56 ± 5.49 |
| Group 3, MC extract ointment 2.5% | 276.22 ± 9.42 |
| Group 4, MC extract ointment 5% | 303.72 ± 9.7* |
| Group 5, MC extract ointment 10% | 319 ± 9.23*,# |

Values are mean ± SEM (n = 6)

* p < 0.05 vs diabetic control

p < 0.05 vs non diabetic control

The animals treated with 2.5% MC extract showed increase in percentage of wound contraction when compared with diabetic control animals, but was not significant. It was 59.32 ± 3.9 and 82.77 ± 5.26 on day 12 and 16, respectively, in test group that received 5% MC extract, which was significant (p < 0.05) compared to the diabetic control group. The rate of wound contraction on day 12 and day 16, in group with 10% MC extract was significantly (p < 0.05) increased to 69.27 ± 4.21 and 88.07 ± 2.39, respectively, when

compared with diabetic control group and the group that received 2.5% MC extract. But there was no significant increase on day 4 and 8, post-operatively (Table 2).

The mean period of epithelialization in nondiabetic control group was 18.34 ± 0.19 days. This was significantly (p < 0.05) longer in diabetic control group (21.86 ± 0.21 days) and test group-1 (20.78 ± 0.54 days) when compared with nondiabetic control group. The mean period of epithelialization was significantly (p < 0.05) reduced in animals treated with 5% MC extract (19.09 ± 0.36 days). The group treated with 10% MC extract showed a decrease in mean period of epithelialization (18.28 ± 0.76 days), which was significant (p < 0.05) when compared with diabetic control and group 3 (Table 1).

The mean hydroxyproline content of granulation tissue in nondiabetic control group was 142.21 ± 2.62 mg/g dry tissue weight; this was significantly (p < 0.05) higher when compared with diabetic control group (115.67 ± 1.83 mg/g), group 3 (119.36 ± 1.16 mg/g) and group 4 (128.51 ± 2.27 mg/g) respectively.

Table 2: Effect of topical *Michelia champaca* flower extract on wound contraction rate, hydroxyproline content and period of epithelialization of excision wound in diabetic rats

| Group, treatment | Wound contraction (%) | | | | POE | Hydroxyproline in mg/g dry tissue weight |
|---|-----------------------|----------------|-----------------|-----------------|-----------------|--|
| | Day 4 | Day 8 | Day 12 | Day 16 | | |
| Group 1, Ointment base (Non-diabetic) | 19.32 ± 2.43 | 44.82 ± 1.76 * | 62.71 ± 3.53 * | 82.46 ± 2.43 * | 18.34 ± 0.19*,† | 142.21 ± 2.62*,†,‡ |
| Group 2, Ointment base (Diabetic control) | 16.21 ± 4.7 | 32.06 ± 2.29 | 46.2 ± 1.92 | 67.22 ± 5.41 | 21.86 ± 0.21 | 115.67 ± 1.83 |
| Group 3, MC extract ointment 2.5% | 18.52 ± 2.58 | 34.72 ± 4.05 | 52.24 ± 2.61 | 74.42 ± 3.64 | 20.78 ± 0.54 | 119.36 ± 1.16 |
| Group 4, MC extract ointment 5% | 20.5 ± 3.24 | 39.19 ± 2.87 | 59.32 ± 3.9* | 82.77 ± 5.26* | 19.09 ± 0.36* | 128.51 ± 2.27*,† |
| Group 5, MC extract ointment 10% | 22.86 ± 1.32 | 42.97 ± 2.07 | 69.27 ± 4.21*,† | 88.07 ± 2.39*,† | 18.28 ± 0.76*,† | 138.05 ± 1.02*,†,‡ |

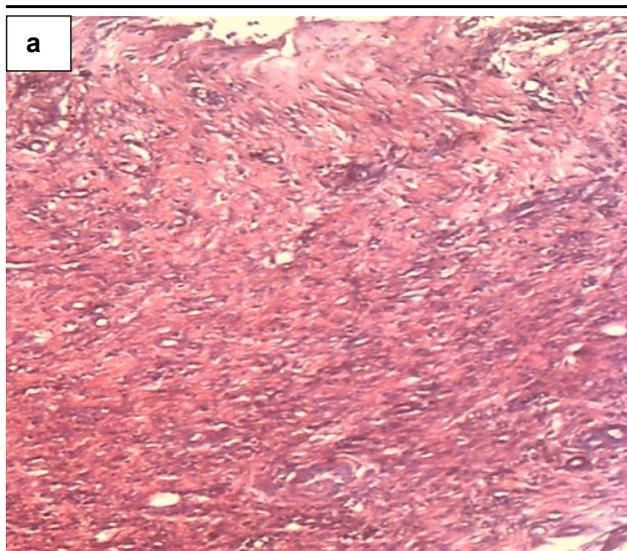
Values are expressed in mean ± SEM, POE = period of epithelialization

* p < 0.05 vs. diabetic control, † p < 0.05 vs. group 3, ‡ p < 0.05 vs. group 4

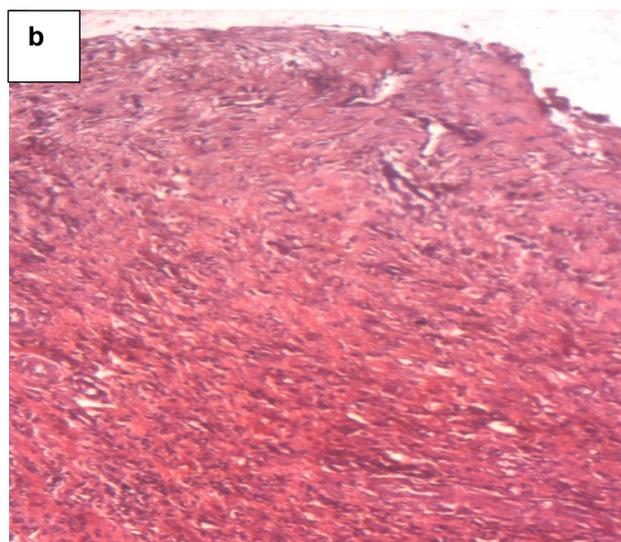
The mean hydroxyproline content in granulation tissue was significantly ($p < 0.05$) higher in group which received 5% when compared with diabetic control group and the group which received 2.5% MC extract. Animals that received 10% MC extract showed significantly ($p < 0.05$) greater levels of mean hydroxyproline content of granulation tissue when compared with diabetic control and group which received 2.5% and 5% MC extract. (Table 2).

Histopathological evaluation of the granulation tissue obtained on day 10 from the experimental animals was performed under a light microscope. Fewer macrophages and fibroblasts were observed in diabetic control group when compared with nondiabetic control group. The diabetic control group also showed poorly formed granulation tissue and sparse distribution of collagen fibers. A significant increase in number of macrophages and fibroblasts was observed. The treated animals also showed a denser distribution and better organization of collagen fibers within the granulation tissue. These changes were more prominent in group that was treated with 10% MC extract (Figure 1).

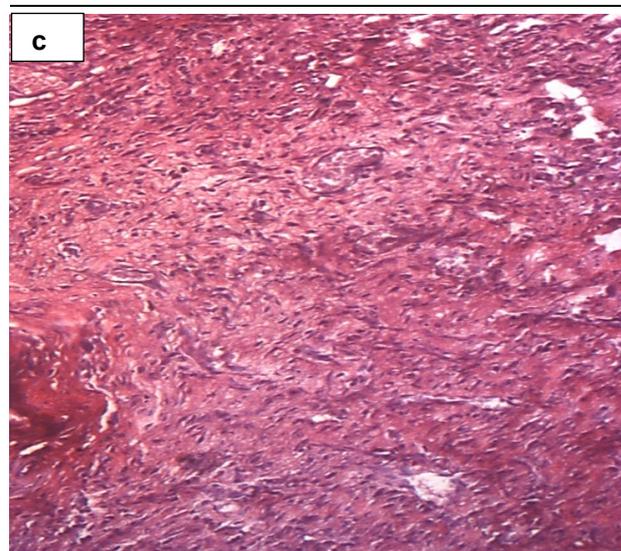
Figure 1: Histology of wound on day 10 in excision wound rats.



(a) Nondiabetic control group shows normal granulation tissue with inflammatory cells and fibroblasts; well laid collagen fibers.



(b) Diabetic control group shows fewer inflammatory cells and fibroblasts; sparse distribution of collagen fibers



(c) MC extract treated skin shows significant increase in number of inflammatory cells and fibroblasts; denser distribution and better organization of collagen fibers. The changes are more evident in group treated with topical 10% MC extract.

DISCUSSION

Diabetes mellitus is one of the factors affecting the normal course of wound healing. Diabetic wound healing is characterized by a delay in cellular infiltration and formation of granulation tissue, decreased wound collagen content, diminished wound tensile strength and prolonged epithelialization time.^[19] Experimental diabetes has been shown to impair wound healing by decreasing collagen concentration and formation of granulation tissue by increasing activities of protease and collagenase.^[20]

It has been suggested that alterations in the wound healing process are present even at the onset of diabetes that can be associated with deficiencies in the defense cells involved in normal wound healing with marked decrease in the production of collagen.^[21]

In our study, the mean breaking strength of the repaired tissue was decreased in the diabetic control animals. Topical preparations of *M. champaca* enhanced the mean breaking strength of the wound. Similar observations were made by another study, where, in dexamethasone suppressed wound model, the mean breaking strength of the incision wound was increased with *M. champaca* (oral, 250 mg/kg), but was not significant.^[6]

The full -thickness wound in diabetic rats is clinically relevant and comparable with impaired wound healing in diabetes mellitus in humans.^[22] In this study, the rate of wound contraction was significantly ($p < 0.05$) reduced in diabetic controls; *M. champaca* extract treated wounds (5% and 10%) showed better rate of contraction. In a previous study, the results were closely similar to the current study. The wound contraction rate was increased significantly with topical application of *M. champaca*, whereas oral dosage showed significant increase in the rate of wound contraction only on 12th day.^[7]

In the present study, topical treatment with *M. champaca* accelerated the wound healing in diabetic animals. The effect was even greater on (5% and 10%MC extract) application. These results are in accordance with the other studies, where the period of epithelialization was reduced in orally and topically treated animals, when dexamethasone suppressed excision^[6] and burn wound models were used.^[7]

The major final product of granulation tissue is collagen, of which, hydroxyproline is a main component. It provides strength and sup-

port, and acts as an indicator of the amount of collagen in a tissue sample. The measurement of hydroxyproline is an index for collagen turnover.^[23]

In the current study, samples were taken for hydroxyproline only from the center of the wound granulation tissue, and this measure represents predominantly, if not exclusively, newly synthesized collagen. There was a significant ($p < 0.05$) reduction in hydroxyproline content of granulation tissue in untreated diabetic rats. Topical *M. champaca* showed greater increase in hydroxyproline content of granulation tissue, which was highest with the 10% ointment. This is consistent with the study that used dexamethasone suppressed wound model, where animals treated orally with *M. champaca* showed greater hydroxyproline content when compared with dexamethasone control group, although not significant.^[6]

In the present study, the histopathological examination of granulation tissue obtained from topically treated rats showed an increase in number of macrophages and fibroblasts. The treated animals also showed a denser distribution and better organization of collagen fibres. Possibly, the constituents like alkaloids, triterpenoids and tannins of *M. champaca* may play a major role in the process of wound healing in diabetic rats. Tannins and triterpenoids are known to promote the wound healing process mainly due to their astringent and antimicrobial properties, which seems to be responsible for wound contraction and increased rate of epithelialization.^[24]

In this study, it was observed that defective wound repair in diabetic rats is associated with reduced cellular infiltration, formation of granulation tissue and collagen synthesis. The loss of collagen observed in diabetes may be due to decreased synthesis or enhanced catabolism of newly synthesized collagen or both.^[25]

Elevated levels of hydroxyproline in the regenerated tissue suggest enhanced collagen synthesis. Collagen not only confers strength and integrity to the tissue matrix but also plays an important role in hemostasis and epithelialization at a later phase of wound healing.^[26] Hence, enhanced collagen synthesis by *M. champaca* in diabetic rats may contribute significantly to healing and also provide necessary strength to the repaired tissue. Since incisional wounds treated with *M. champaca* showed greater breaking strength, it may be speculated that it not only increases collagen synthesis, but also aids in cross-linking of the protein, as indicated in the other study.^[8] Inhibition of collagenases can also increase the rate of wound filling by granulation tissue and the amount of collagen.

M. champaca may stimulate cellular proliferation and migration through a yet unknown mechanism. This was evident in the histological studies. The treated group of diabetic rats showed an increase in macrophages and fibroblasts on day 10 after wound creation, which indicates that *M. champaca* may stimulate macrophage infiltration into the wound environment, collagen synthesis and re-epithelialization. The macrophages play an important role in activation and recruitment of other cells like fibroblasts via mediators such as TGF- β , VEGF, PDGF, IGF-1, EGF and lactate. These mediators regulate cell proliferation, matrix synthesis, and angiogenesis.^[27]

Diabetes mellitus involves oxidative stress, which results in production of free radicals that in turn cause tissue damage and delay wound healing. There have been several studies which have reported antioxidant properties of *M. champaca*,^[28] which suggest the beneficial effect of this plant in wound healing in diabetics.

Wounds in diabetics have a higher propensity to become infected, which may impede

the progress or completion of healing. There have been several studies which have demonstrated the antimicrobial property of flower extracts of *M. champaca*,^[28] which may play a role in enhanced wound healing in the treated animals.

Since the topical administration of ethanolic extract of *Michelia champaca* flowers significantly enhanced the wound healing process, it could be made use of clinically, as a supportive therapy to treat wounds in diabetic patients.

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REFERENCES

1. Robson MC. Wound infection. A failure of wound healing caused by an imbalance of bacteria. *Surg Clin North Am* 1997;77:637-50.
2. Snyder RJ. Treatment of nonhealing ulcers with allografts. *Clin Dermatol* 2005;23:388-95.
3. Varier PS. Indian medicinal plants. 1st ed. Vol 4. Chennai: Orient Longman Pvt.Ltd; 2003. p.33-5.
4. Rajagopalan PM. Siddha medicine. Madurai: Siddha Maruthuva Gurukulam; 2000.
5. Vimala R, Nagarajan S, Alam M, Susan T, Joy S. Antiinflammatory and antipyretic activity of *Michelia champaca* Linn., (white variety), *Ixora brachiata* Roxb. and *Rhynchosia cana* (Willd.) D.C. flower extract. *Indian J Exp Biol* 1997;35:1310-14.
6. Dwajani S, Shanbhag T. *Michelia champaca*: Wound healing activity in immunosuppressed rats. *The Internet Journal of Alternative Medicine* 2008;7:no.2.
7. Shanbhag T, Kodidela S, Shenoy S, Amuthan A, Kurra S. Effect of *Michelia champaca* Linn flowers on burn wound healing in Wistar rats. *International Journal of Pharmaceutical Sciences Review and Research* 2011;7:112-5.

8. Bairy KL, Kumar SMC, Savin CG, Kumar KN, Avinash M. Effect of different formulations of silver sulphadiazine cream on experimentally induced burn wound healing. *Res J Pharm Biol Chem Sci* 2012; 3:884-9.
9. Babu G, Sanjeeva, Bairy KL. Effect of *Tridax procumbens* on burn wound healing. *Indian Drugs* 2003;40:488-91.
10. Low-Dose Streptozotocin Induction Protocol. Animal Models of Diabetic Complications Consortium Protocols [internet]. 2003 Apr 9 [cited 2012 Sep 8]. Available from: <http://www.diacomp.org/shared/showFile.aspx?doctypeid=3&docid=19>
11. Vogel HG, Muller G, Herling AW. Methods to induce experimental diabetes mellitus. In: Vogel HG, Vogel WH, Scholkens BA, Sandow J, Muller G, Vogel WF, editors. *Drug Discovery and Evaluation: Pharmacological Assays*. 2nd ed. New York: Springer-Verlag Berlin Heidelberg; 2002. p.948-54.
12. Gutierrez RMP, Vargas SR. Evaluation of wound healing properties of *Acalypha langiana* in diabetic rats. *Fitoterapia* 2006;77:286-9.
13. Ehrlich HP, Hunt TK. The effects of cortisone and anabolic steroids on the tensile strength of healing wounds. *Ann Surg* 1969;170:203-6.
14. Lee KH. Studies on the mechanism of action of salicylate. II. Retardation of wound healing by aspirin. *J Pharm Sci* 1968;57:1042-3.
15. Morton JP, Malone MH. Evaluation of vulneray activity by an open wound procedure in rats. *Arch Int Pharmacodyn Ther* 1972;196:117-26.
16. Kumar MS, Kirubanandan S, Sripriya R, Sehgal PK. Triphala promotes healing of infected full-thickness dermal wound. *J Surg Res* 2008;144:94-101.
17. Gutierrez RMP, Solis RV. Wound healing properties of triterpenes from *Buddleia scordioides* in diabetic rats. *Pharm Biol* 2008;46:647-53.
18. Fujii A, Kobayashi S, Kuboyama N, Furukawa Y, Kaneko Y, Ishihama S, et al. Augmentation of wound healing by royal jelly (RJ) in streptozotocin-diabetic rats. *Jpn J Pharmacol* 1990;53:331-7.
19. Pelegrinelli FF, Thirone AC, Gasparetti AL, Araujo EP, Velloso LA, Saad MJ. Early steps of insulin action in the skin of intact rats. *J Invest Dermatol* 2001;117:971-6.
20. Goodson WH 3rd, Hunt TK. Wound collagen accumulation in obese hyperglycemic mice. *Diabetes* 1986;35:491-5.
21. Spanheimer RG, Umpierrez GE, Stumpf V. Decreased collagen production in diabetic rats. *Diabetes* 1988;37:371-6.
22. Gulcelik MA, Dincer H, Sahin D, Demir OF, Yenidogan E, Alagol H. Glucan improves impaired wound healing in diabetic rats. *Wounds* 2010;22:12-6.
23. Ya C, Gaffney S, Lilley TH, Haslam E. Carbohydrate-polyphenol complexation. In: Hemingway RW, Karchesy JJ, editors. *Chemistry and significance of condensed tannins*. 2nd ed. New York: Plenum Press; 1989. p.134-45.
24. Lien YH, Tseng MM, Stern R. Glucose and glucose analogs modulate collagen metabolism. *Exp Mol Pathol*. 1992;57:215-21.
25. Clark RAF. Wound repair: overview and general considerations. In: Clark RAF, editor. *The molecular and cellular biology of wound repair*. 2nd ed. New York: Plenum Press; 1996. p.1-48.
26. DiPietro LA. Wound healing: the role of the macrophage and other immune cells. *Shock* 1995;4:233-40.
27. Zabel DD, Feng JJ, Scheuenstuhl H, Hunt TK, Hussain MZ. Lactate stimulation of macrophage-derived angiogenic activity is associated with inhibition of Poly (ADP-ribose) synthesis. *Lab Invest* 1996;74:644-9.
28. Wei LS, Wee W, Siong JYF, Syamsumir DF. Characterization of antimicrobial, antioxidant, anticancer property and chemical composition of *Michelia champaca* seed and flower extracts. *S J Pharm Sci*. 2011; 4:19-24.
