www.sphinxsai.com

PharmTech

International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.1, No.4, pp 1267-1270, Oct-Dec 2009

Wound healing activity of *Terminalia Chebula* in experimentally induceddiabetic rats

Manish Pal Singh[1]* and Chandra Shekhar Sharma[2]
[1]Department of Pharmacology, Shrinathji Institute of Pharmacy, Nathdwara, Rajsamand, India
[2]Department of Pharmaceutical Chemistry, B. N. College of Pharmacy, Udaipur, Rajasthan, India
***Email ID : manish_bn@yahoo.co.in**

ABSTRACT: The hydroalcoholic extract of *T.Chebula* fruit was evaluated for its wound healing activity in alloxan induced diabetic rats using excision and dead space wound models. Extract treated animals exhibited 82% reduction in the wound area when compared to controls which was 40%. The extract treated wounds were found to epithelize faster as compare to controls. The wet and dry granulation tissue weight content was increased significantly when compared to controls. *T.Chebula* promotes significant wound healing in diabetic rats and further evaluation of this activity in humans is suggested.
KEYWORDS: *T.Chebula*, alloxan, excision wound model and dead space wound model.

INTRODUCTION

Diabetic wounds are slow, non-healing wounds that can persist for weeks despite adequate and appropriate care. Such wounds are difficult and tough to manage[1]. Though the exact pathogenesis of poor wound healing in diabetic wounds is not clearly understood, evidence from studies involving both human and animal reveal several abnormalities in the various phases of the wound healing process[2-3].

Terminalia Chebula has been extensively used in ayurveda, unani & homoeopathic medicine and has become cynosure of modern medicine. The Sanskrit name 'Haritaki' is rich with meaning, reffering to the yellowish dye (harita) that contains the god Siva (Hari, i.e. the Himalayas) and that it cures (harayet) all the diseases[4]. Its other commonly used Sanskrit name, Abhaya, refers to the 'fearlessness' it provides in the face of the disease. According to Indian mythology, this plant originated from the drops of ambrosa (Amrita) which fell on the earth when Indra was drinking it[5].

T. Chebula possesses a wide variety of activities like antimicrobial[6], antioxidant[7], antiviral[8], anticarcinogenic[9], hypocholesterolemic[10], radioprotective[11], antispasmodic & antipurgative[12].

The present study has been undertaken to examine the wound healing activity of the fruit extract of *T.Chebula* in experimentally induced excision and dead space wounds in diabetic rats.

Corresponding author:
Manish Pal Singh
Lecturer
Department of Pharmacology
Shrinathji Institute of Pharmacy, Nathdwara, Rajsamand, India
***Email ID :** manish_bn@yahoo.co.in
Phone No. : +91-98287-83211
Fax No. : +91-294-2413182

MATERIALS AND METHODS

Preparation of fruit extract- 100 gm of powdered drug was soaked in 250 ml of aqueous alcoholic solution (50%) for 24 hrs followed by cold maceration for further 48 hrs with occasional shaking. The mixture was filtered using muslin cloth followed by removal of excess of solvent by means of Rotatory evaporator.

Animals- The study was approved by Institutional Animal Ethical Committee, B. N. College of Pharmacy, Udaipur, Rajasthan. Healthy Sprague Dawely rats weighing 150-180 g were maintained on the standard rodent fed and water *ad libitum*. The excision & dead space wound models were used to evaluate wound healing activity of *T.Chebula* extract. The animals were randomly distributed into five groups of 6 each in

excision and four groups of 6 each in dead space wound models.

Inductions of diabetes- Animals of diabetic groups were weighed and their fasting blood glucose levels were determined before inducing diabetes. Animals showing very low or high glucose levels were replaced. The animals were injected with a single dose of Alloxan monohydrate (120 mg/kg, Sigma) in normal saline (freshly prepared) by i.p. route. Control animals were injected with normal saline. Fasting blood glucose level was measured 3 days later to confirm the diabetic status of the animals. For blood glucose measurement, the blood was drawn by tail vein.

Excision wound model- Animals were anaesthetized with diethyl ether by open mask method and shaved on both sides of the back with an electric clipper. The area of wound to be created was outlined on the back of the animals with methylene blue using a stainless steel stencil. The entire wound was left open. Animals were closely observed for any infection and those which showed signs of infection were separated, excluded from the study and replaced. Animals were divided into five groups of 6 each. The normal controls (group 1) were applied with ointment base two times a day, experimental controls (group 2) were applied 20% w/w ointment in soft paraffin base extract of *T.Chebula* two times a day, diabetic controls (group 3) were applied with ointment base two times a day, diabetic experimental rats (group 4) were applied 20% w/w ointment in soft paraffin base extract of *T.Chebula* two times a day and the positive controls received an application of mupirocin ointment two times a day (group 5). The treatment was done topically in all the cases. Wound areas were measured on days 1, 5 and 11 for all the groups using a transparency sheet and a permanent marker. Recording of the wound areas were measured on graph paper[13-14].

$$\% \text{ Wound closure} = \frac{\text{Wound area on day(0)} - \text{Wound area on day(n)}}{\text{Wound area on day 0}} \times 100$$

Where , n= numbers of days (0^{th}, 5^{th} and 11^{th})

Dead space wound model- Dead space wounds were inflicted by implanting sterile cotton pellets (10 mg each), one on either side in the groin and axilla on the ventral surface of each rat. The animals were divided into four groups of 6 each. The normal controls (group 1) were provided plain water orally, experimental controls (group 2) were given the extract orally in a dose of 200 mg/kg for 10 days, diabetic controls (group 3) were given plain water orally and diabetic experimental rats (group 4) were given extract orally at a dose of 200 mg/kg for 10 days. On the 10^{th} post-wounding day, the granulation tissue formed on the implanted cotton pellets was carefully removed under anesthesia. After noting the wet weight of the granulation tissue, the tissue was dried at 60^0C for 12hr and weight was recorded[15].

Statistical analysis- The means of wound area measurements between groups at different time intervals were statistically analyzed by one-way analysis of Variance (ANOVA) followed by Dunnett's test as post hoc test. P value <0.05 was considered statistically significant using Graph Pad software.

RESULTS AND DISCUSSION

The excision wound model was carried out to study the topically applied *T.Chebula* fruit extract on wound healing and contraction, Increase in the wound healing activity was observed in fruit extract treated rats. On 5^{th} day, animals of group 2 showed greater percentage of wound contraction when compared with the animals of group. In diabetic animals also percentage wound contraction was greater in extract treated group 4 than in control group 3 animals. The same pattern was observed on 11^{th} day also. The wound contraction results of extract-treated animals were comparable with positive controls as shown in Table1.

The dead space wound model was used to study difference in matrix synthesis between drug treated and control groups. Oral administration of the fruit extract appears to increase the mass of granuloma in both normal as well as diabetic animals. However the dry granuloma weight is decreased by the extract treatment, in non diabetic animals. In diabetic animals the dry granuloma mass is increased by extract treatments. The normal treated group 2 had greater wet/dry ratio than any other group as shown in Table 2.

From these animal studies it can be concluded that significant increase in the wound healing activity was observed in fruit extract treated rats. In excision wound model, animals of groups 2 and 4 showed a decrease in the epithelialization period and increased percentage of wound contraction when compared with the animals of groups 1, 3 and 5 (Table 1). On day 11, the extract-treated animals (groups 2 and 4) showed wound contraction by 82% compared with 40% in wounds of the control groups (groups 1 and 3). The wound contraction results of extract-treated animals were comparable with positive controls (86%).

In the dead space wound model (Table 2), the extract-treated animals in groups 2 and group 4 showed significant increase in the dry and wet weight of the granulation tissue in the animals treated with the extract was observed. Overall the weights of the animals did not differ for any of the study groups.

The present study demonstrates that *T.Chebula* extract applied topically promotes healing of wound contraction in alloxan induced diabetic rats where healing is delayed. These preliminary results further suggest that *T.Chebula* facilitates healing by increasing the rate and extent of wound closure.

Table 1: Wound healing activity of the T.Chebula in alloxan induced diabetic rats (excision wound model)

Parameter Wound Area (mm^2)	Group-1 (Normal Control)	Group-2 (Normal Treated)	Group-3 (Diabetic Control)	Group-4 (Diabetic Treated)	Group-5 (Positive Control)
Day 1	218±1.880	218.7±1.764	217.8±1.537	216.5±1.310	217±1.770
Day 5	45±1.461 (22%)	137.2±2.151** (64%)	42.5±0.7638 (20%)	133.8±1.352** (61%)	145.3±1.726** (68%)
Day 11	92.33±1.256** (42%)	184.2±1..815** (87%)	82.33±1.116** (39%)	165.7±1.892** (77%)	186.2±1.939** (86%)

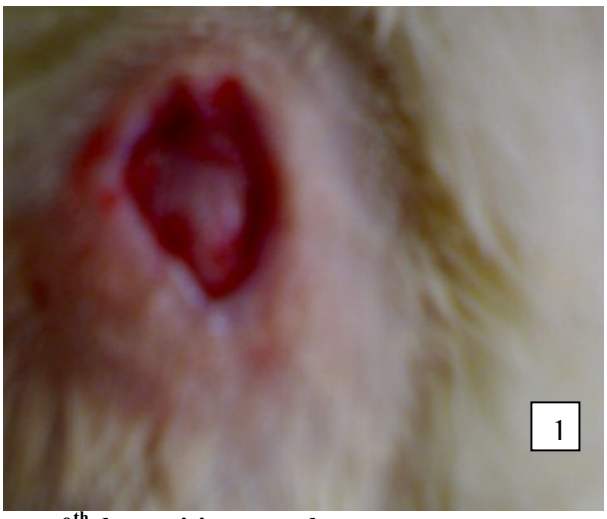
The values are showed as mean ± SE from 6 animals in each group,
** shows significant as compared to normal control ($p<0.01$).

Table 2: Wound healing activity of the T.Chebula in alloxan induced diabetic rats (dead space wound model)

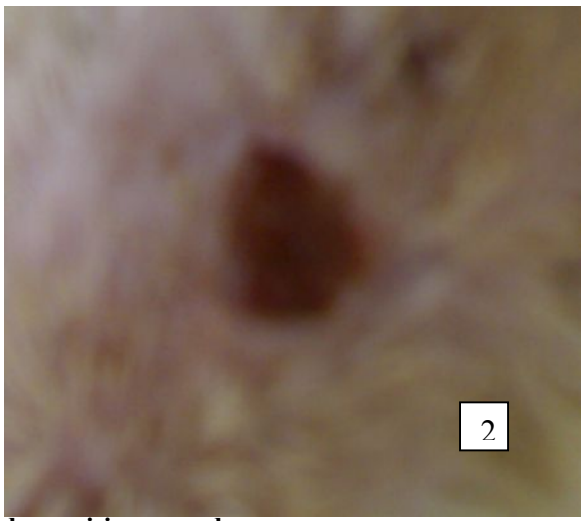
Parameter	Group-1 (Normal Control)	Group-2 (Normal Treated)	Group-3 (Diabetic Control)	Group-4 (Diabetic Treated)
Wet granulation weight (mg/100 g rat)	99.67±8.168	152.8±17.54**	75.17±3.381	103.7±7.116
Dry granulation weight (mg/100 g rat)	38.17±4.262	35.83±6.134	33.83±0.9458	40.5±9.787

The values are showed as mean ± SE from 6 animals in each group,
** shows significant as compared to normal control ($p<0.01$).

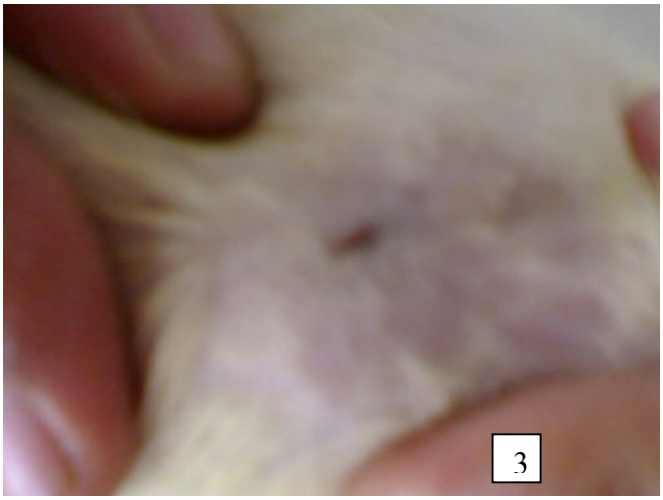
Figure 1: Photographs showing various stages of wound healing activity of *T.Chebula* extract in alloxan induced diabetic rats. (Excision wound model)



0^{th} day excision wound



5^{th} day excision wound



11th day excision wound

ACKNOWLEDGEMENTS

Authors are thankful to Department of Dravyaguna Vigyana, National Institute of Ayurveda, Jaipur, Rajasthan for the plant authentification.

REFERENCES

1. Falanga V., The chronic wound: Impaired wound healing and solutions in the context of wound bed preparation, Blood Cells Mol. Dis., 2004, 32, 88.
2. Singer A.J. and Clark R.A., Cutaneous wound healing, N. Eng. J. Med., 1999, 341, 738.
3. Goodson W.H. and Hunt T.K., Studies of wound healing in experimental diabetes mellitus, J. Surg. Res., 1977, 22, 221.
4. Das B., Materia medica of ayurveda, B. Jain Publisher, New Delhi, 1991, 8.
5. Srikanthmurthy K.R., Bhavaprakasha of bhavamishr, Krishnanda Academy, Varanasi, 2000, 160.
6. Sato Y., Oketani H., Singyouchi K., Ohtsubo T., Kihara M., Shibata H. and Higuti P., Extraction and purification of effective antimicrobial constituents of *Terminalia chebula* Retz. against methicillin- resistant *Staphylococcus aureus*, Bio. Pharm. Bull., 1997, 20, 404.
7. Cheng H.Y., Lin T.C., Yu K.H., Yang C.M. and Lin C.C., Antioxidant and free radical scavenging activities of *Terminalia chebula*, Bio. Pharm. Bull., 2003, 26, 355.
8. Jeong A.H.N., Kim C.Y., Lee J.S., Kim T.G., Kim S.H., Lee C.K., Lee B., Shim C.G., Hoon H. and Kim J., Inhibitors of HIV-1 integrase by galloyl glucose from *Terminalia chebula* and flavonol glycoside gallates from *Euphorbia pekinensis*, Planta Medica, 2002, 68, 459.
9. Hushum S.M., Harkonen P. and Pihlaja K., Inhibition of cancer cell growth by crude extract and phenolics of *Terminalia chebula* fruit, J. Ethnopharmacol., 2002, 81, 336.
10. Thakur C.P., Thakur B., Singh S. and Sinha S.K, The ayurvedic medicines haritaki, amla and bahira reduced cholesterol induced atherosclerosis in rabbits, Int. J. Cardiol., 1988, 21, 175.
11. Gandhi N.M. and Nayar C.K.K., Radiation protection by *Terminalia chebula* some mechanistic aspects, Mol. Cell. Biochem., 2005, 48, 277.
12. Miglani B.D., Sen P. and Sanyal P.K., Purgative action of an oil obtained from *Terminalia chebula*, Indian J. Med. Res., 1971, 52, 283.
13. Shivananda B.N., Lexley P.P. and Dale M., Wound healing activity of *Carica Papaya L.* in experimentally induced diabetic rats, Indian J. Exp. Bio., 2007, 45, 743.
14. Senthil M.K., Kirubanandan S. and Sripriya R., Triphala promotes healing of infected full thickness dermal wound, J. Surg. Res., 2008, 22, 89.
15. Turner R.A, Inflammatory agent in screening methods of pharmacology, Academic Press, New York, 2nd Edi., 1965, 475.

*****