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RESEARCH ARTICLE

PHARMACOGNOSY

WOUND HEALING ACTIVITY OF THE ETHANOLIC EXTRACT OF TERMINALIA CHEBULA RETZ.

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ABSTRACT

The wound healing activity of ethanolic extract of fruit of *Terminalia chebula* evaluated on excision and incision model, in albino rats, in the form of an ointment with two concentrations (5% and 10% w/w ointment of bark extract in simple ointment base).Both concentrations of the ethanolic extract showed significant response in both the wound types tested when compared with the control group. Nitrofurazone ointment (0.2%w/w) used as standard.

KEY WORDS

Terminalia chebula,Ethanol extract,Nitrofurazone,wound healing.

INTRODUCTION

In tribal areas different crude drugs extract used to treat various skin disorders including wound. Wound healing process involve several steps, which involves coagulation, formation of granulation tissue, collagination and aquisation of wound strength. During the formation of new tissue,endothelial cells proliferate and form new blood vessels. . *Terminalia chebula* Retz. belonging to the family-Combretaceae, commonly known as harde, is a deciduous tree found throughout the Indian forests and plains. The tree is about 15-25 m. in height and 1.5-2.5 m. in girth. Harde is drupe, brown in color. It is ovate longitudinallywrinkled, 2 to 3.5 cm. Long and 1.3 to2.5 cm. Broad. Fruit has 5 to 6 ribs. Fruit is astringent, antiseptic, rejuvenative, tonic, anthelmintic and laxative. It is used in chronic ulcer wound, piles and stomatitis. [1-3].
Fruit contain about 30-32% of tannin, free tannic acid, gallic acid and ellagic acid, glucose and sorbitol[3,4].
Terminalia chebula has been used in folk medicine for the treatment of skin diseases and wound. A survey of literature revealed that no systematic approach has been made to study the wound healing activity of this plant. Thus the present study was undertaken to assess the effect of this indigenous plant on different parameters related to wound healing in rats.

MATERIALS AND METHODS

Plant material I- Fruit of *Terminalia chebula* obtained from Yucca enterprises,Mumbai, were authenticated and identified by Dr.A.B.Sheerwani. (Retd. Prof. and Head), Deptt. of Botany, Holkar Science College, Indore. A voucher specimen has been deposited in our laboratory for further reference.

Preparation of extract - Powdered fruit were soxhlet-extracted with 90% ethanol. The ethanolic extract was evaporated in vacuo and residue(yield:30%w/w).Ethanolic extract was subjected to tests of Kokate[5] . The phytochemical screening revealed the presence of tannins and glycoside.
Preparation of drug formulation-Two types of ointment formulations with different concentration of the extract were prepared *viz*. 5%(w/w) ointment, where 5g. of extract was incorporated in 100g. of simple ointment base;10%(w/w) ointment where,10g. of extract incorporated in 100g.of simple ointment base. Nitrofurazone ointment(0.2 w/w) was used as standard drug for comparing the wound healing potential of the extract in different animal model.

Animals - Healthy Wistar albino rats of either sex weighing 150-200gm.were used. They were kept in a standard environment condition and fed with rodent diet and water *ad libitum.* The experimental protocol have been approved by institutional animal ethical committee.
wound healing activity-The wound healing studies were carried out using ether anaesthetized rats in two different wound model at two different concentrations(5% and 10%w/w).
Incision wound -In incision wound model four groups (The group I was considered as control, the group II served as the reference standard and treated with 0.2%w/w Nitrofurazone ointment. The group III animals were treated with the 5%w/w ethanolic extract and the group IV animals were treated with 10%w/w ethanolic extract of fruit of *T. chebula)* of

animals containing six in each group. Paravertebral incision of 6cm. long were made on either side of the vertebral column of the rat .Care was taken to see that incision was at least 1cm. lateral to vertebral column. The wounds were closed with interrupted sutures of 1cm. apart. The animals were caged individually. The sutures were removed on 8th post wounding day. The tensile strength of the wound was measured on 10th post wounding day[6].

Excision wound **-** In excision wound four groups(The group I was considered as control, the group II served as the reference standard and treated with 0.2%w/w Nitrofurazone ointment.The group III animals were treated with the 5%w/w ethanolic extract and the group IV animals were treated with 10%w/w ethanolic extract of fruit of *T. chebula)* of animals containing six in each group.A circular piece of full thickness (approx.500 mm^2) was cut off from a predetermined area on the back of the rat.wounds were traced on $1mm^2$ graph paper on the day of wounding and subsquently on alternate days until healing was complete. Changes in wound area were calculated, giving an indication of the rate of wound contraction. Number of days required for falling of the eschar without any residual raw wound gave the period of epithelization[7].The ointment of the fruit extract ,standard drug and simple ointment was applied to the wound twice daily, until recovery to the respective groups of animals.

Statistical analysis-The results are expressed as mean ±SE of six animals in each group.The data were evaluated by students t-test and the values of $P \leq 0.001$ were considered statistically significant.

RESULTS

It was observed that the wound healing contracting ability of the extract ointment in different concentrations was significantly greater than that of the control (i.e. simple ointment treated group). The 10% (w/w) extract ointment treated groups showed significant wound healing from the fourth day onwards, which was comparable to that of the standard drug, i.e. nitrofurazone ointment treated group of animals. The wound closure time was lesser, as well as the percentage of wound contraction was much more with the 10% w/w extract ointment treated group (18±1 days for 100% contraction which was almost similar to that of the nitrofurazone treated group). The 5%(w/w) extract ointment treated group of animals showed significant wound contraction from the eighth day onwards and achieved 100% with the wound closure time of 20 ±2 days.

In incision wound model the measurement of the effect of the extract and standard drug on the tensile strength is shown in Table 2. The tensile strength of the 10% extract treated group and the nitrofurazone ointment treated group were comparable to each other. The 5% extract ointment treated group showed a lesser but significant increase in the tensile strength compared to the control group. Thus both concentrations of the

extract as well as the standard drug showed a significant increase in tensile strength in the 10 days old wound. The results of the present study revealed that both concentration (5% and10%w/w) of ethanolic extract of *T.chebula* fruit have significant wound healing activity in both incision as well as excision wound models.

Table II

Effect of ethanolic extract of fruit of Terminalia chelula on tensile strength of incision wound

Treatment groups	Tensile strength(g)
Control	310±4.6
Nitrofurazone	564±1.8*
Extract(10%)	529±4.2*
Extract(5%)	445±4.8*

Values are mean ±SE *P<0.001 vs control n=6 animals in each group

Table I

Effect of ethanolic extract of fruit of Terminalia chelula on % wound closure of excision wound model

Group	4th Day	8th Day	12th Day	16th Day	Period of epithelization in days
Control	15.82±0.68	27.21±1.02	48.21±1.80	68.53±2.60	24
Nitrofurazone	35.28±0.15	76.80±0.19	89.81±0.58	97.11±048	18
Extract(10%)	32.44±1.01	74.22±1.24	82.32±2.36	90.34±2.10	18*
Extract(5%	20.12±1.02	34.86±1.82	60.94±2.78	81.56±2.32	20

Values are mean ±SE *P<0.001 vs control n=6 animals in each group

DISCUSSION

Wound healing is a fundamental response to tissue injury that results in restoration of tissue integrity. This is mainly achieved by the synthesis of the connective tissue matrix. Collagen is a major protein of the extracellular matrix and is the component that ultimately contributes to wound strength.Tannins promote the wound healing through several cellular mechanism; chelation of the free radicals and reactive species of oxygen, promoting contraction of the wound and increasing the formation of capillary vessels[8] and fibroblasts and including keratinocyte proliferation , but do not act on the differentiation towards cornified cells [9]. Similar findings have been reported with the extracts of the plants containing tannins by earlier workers[10-12]. However, our results revealed that tannins are one of the important phytoconstituents responsible for wound healing mainly due to their astringent and antimicrobial property.

Hence, it can be inferred that the wound healing activity of the fruit of the plant *T.chebula* is due to its high tannin content, which seems to be responsible for wound contraction and increased rate of epithelization

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