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Studies on *in vivo* Wound Healing Activity of *Cassia fistula* Linn. Leaves (Leguminosae) in Rats

T. Bhakta, Pulok K. Mukherjee[a], Kakali Mukherjee, M. Pal, and B.P. Saha*

Department of Pharmaceutical Technology, Faculty of Engineering and Technology, Jadavpur University, Calcutta-700 032, India
[a]JSS College of Pharmacy Rocklands, P.O. Box 20 Ootacamund-643 001, India

Abstract – *Cassia fistula* commonly known as Sundali was selected to evaluate its wound healing potentials based on traditional use and literature references. Methanol extract of *C. fistula* leaves were examined for its wound healing property in the form of an ointment in two types of wound models in rats : i) Excision wound model and ii) Incision wound model. The ointment of the leaf extract of two different concentrations (5% and 10% w/w ointment of leaves extract in simple ointment base) responded significantly in both models of wounds tested. The results were also comparable to that of standard drug, nitrofurazone in terms of wound contraction ability, epithelisation period, tensile strength and regeneration of tissue at wound area.

Key words – *Cassia fistula*; methanol extract; wound healing activity; nitrofurazone.

Introduction

Cassia fistula Linn. is a deciduous middle-sized tree, indigenous to India and often cultivated as an ornamental plants (Deb, 1981 and Wallis, 1985). In many parts of India this plant is used by traditional medical practitioners for the treatment of various diseases; leaves are used in ringworm, as purgative and many other different diseases (Chopra *et al.*, 1956). The antipyretic, antifungal and antibacterial activities of this plant have already been reported (Anis *et al.*, 1986, Ramakrishna *et al.*, 1977). In Ayurvedic system of medicine this plant is used in haematemesis, pruritis, leucoderma, diabetes and many other ailments (Asolker *et al.*, 1992; Alam *et al.*, 1990). The wound healing potentials of *Leucas lavandulaefolia* Rees has been reported from our laboratory by Saha *et al.* (1997). Based on its use in wound healing in traditional practices the present study was undertaken to evaluate its wound healing activity and thereby to substantiate this claim, which is being reported hereunder.

*Author for correspondence.

Experimental

Plant material – *Cassia fistula* leaves were collected freshly from Agartala, Tripura, India. It was identified by Botanical Survey of India, Sibpur, Howrah. A voucher specimen has been kept in our laboratory for future references. The leaves were dried under shade, pulverized by a mechanical grinder, passed through 40 mesh sieve and stored in

a closed vessel for future use.

Extract and standard used – The powdered leaves of *Cassia fistula* were extracted with methanol using soxhlet extraction apparatus. This methanol extract was then concentrated and dried under reduced pressure. The semi-solid mass (methanol free) thus obtained was used for the experiment. The yield was 12.2% w/w with respect to dry powdered material. Two types of formulations were prepared from the extract (i) 5% (w/w) ointment where 5 g extract was incorporated in 100 g of simple ointment base B.P. (Anonymous, 1953); (ii) 10% (w/w) ointment where 10 gm of extract was incorporated in 100 g of simple ointment base B.P. 0.2% (w/w). Nitrofurazone ointment (Smith Kline Beechem Pharmaceuticals India Ltd., Bangalore) was used as standard drug for comparing the wound healing potential of the extract.

Animal used – Wistar albino rats (150-180 g) purchased from M/S B.N. Ghosh & Co. Ltd., Calcutta were selected to carry out the experiment. Six rats were taken for each group. The rats were used after an acclimatization period of 7 days to the laboratory environment. They were housed in standard metal cages and provided with food and water *ad libitum*.

Excision wound model (Udupa *et al.*, 1994) – Five groups of animals containing six in each group were anaesthetised by open mask method with anaesthetic ether. The rats were depilated on the back. One excision wound was inflicted by cutting away 500 mm^2 full thickness of skin of a predetermined area. Rats were left undressed to the open environment. Then the drug i.e. the reference standard (0.2% w/w nitrofurazone ointment), simple ointment B.P. (Anonymous, 1953); *Cassia fistula* methanol extract ointment (5% w/w and 10% w/w) were administered till the wound was completely healed (Chatterjee *et al.*, 1993). This model was used to monitor wound contraction and epithelisation time. Wound contraction was calculated as percent reduction in wound area. The progressive changes in wound area were monitored planimetrically by tracing the wound margin on a graph paper every alternate day. To determine the changes in healing of wound measurement of wound area on graph paper was expressed as unit (mm^2).

Incision wound model – Five groups of animals containing six in each group were anaesthetised and two paravertebral long incisions were made through the skin and cutaneous muscles at a distance of about 1.5 cm from midline on each side of the depilated back of rat. Full aseptic measures were not taken and no local or systemic antimicrobials were used throughout the experiment (Udupa *et al.*, 1994; Saha *et al.*, 1997). All the groups were treated in the same manner as mentioned in case of excision wound model. No ligature was used for stitching. After the incision was made, the parted skin was kept together and stitched with black silk by 0.5 cm apart and surgical thread (No : 000) and curved needle (No : 11) were used for stitching. The continuous threads on both wound edges were tightened for good adoptation of wound. The wound was left undressed. The ointment of extract, standard drug (nitrofurazone ointment) and simple ointment B.P. (Anonymous, 1953) was applied to the wound twice daily until complete recovery, to the respective groups of animals.

Tensiometer – It consists of a 6×12 inch wooden board with one arm of 4 inch long, fixed on each side of the possible longest distance of the board. The board was placed at the edge of a table. A pulley with bearing was mounted on the top of one arm. An alligator clamp with 1 cm width was tied on the tip of the another arm by a fishing line (20 lb test monofilament) in such a way so that the clamp could reach the middle of the board. Another alligator clamp was tied on a longer fishing line with 1 litre polyethylene bottle on the other end.

Tensile strength of wound represents the promotion of wound healing. Usually wound healing agents promote the gaining of tensile strength. Tensile strength (the force required to open the healing skin) was used to measure the amount of healing. The instrument used for this purpose is called as Tensiometer, which is explained as above. This was designated on the same principle as the thread tested in textile industry. One day before performing the experiment (measurement of tensile strength) the sutures were removed from the stitched wounds of rats after recovery and tensile strength was measured as follows.

Determination of tensile strength – The sutures were removed on 9th day of wounding and the tensile strength was measured on 10th day. Extract ointments along with simple ointment (control) and nitrofurazone ointment (standard) were administered throughout the period, twice daily for 9 days. On 10th day again the rats were anaesthetised and each rat was placed on a stack of paper towels on the middle of the board. The amount of the towels could be adjusted in such a way so that the wound was on the same lebel of the tips of the arms. The clamps were then carefully clamped on the skin of the opposite sides of the wound at a distance of 0.5 cm away from the wound. The longer pieces of the fishing line were placed on the pulley and finally to polyethylene bottle and the position of the board was adjusted so that the bottle receive a rapid and constant rate of water from a large reservoir, until the wound began to open. The amount of water in polyethylene bag was weighed and considered as tensile strength of the wound. The mean determinations were made on both sides of the animals and were taken as the measures of the tensile strength of the wound. The tensile strength of the extract and nitrofurazone ointment treated wounds were compared with control. Tensile strength increment indicates better wound healing promotions of the applied drug.

Results and Discussion

The progress of wound healing in excision wound method of the *C. fistula* leaf extract ointment (5% w/w and 10% w/w) treated groups, simple ointment (control) treated group and nitrofurazone (standard drug) treated group of animals have been shown in Table 1. The measurement of the tensile

Table 1. Efects of *Cassia fistula* extract and nitrofurazone on wound healing by excision wound method

Wound area (mm^2) (mean±SE) and percentage of wound contraction

Post wounding days	Simple ointment	Nitrofurazone ointment (0.2% w/)	Extract ointment (5% w/w)	Extract ointment (10% w/w)
0	530±7.8	512±10.9	520±10.8	515±11.8
2	510±8.7(3.77)	438±11.2(26.17)	486±9.7(6.53)	466±10.3(9.51)
4	465±11.5[a](12.26)	315±11.5[a](38.47)	404±11.8[a](22.30)	330±9.5[a](35.92)
6	415±9.6[a](21.69)	256±10.2[a](50.0)	286±10.5[a](45.0)	288±9.2[a](44.07)
8	404±7.5[a](23.77)	189±10.4[a](63.08)	210±11.3[a](59.61)	195±8.6[a](62.13)
10	373±8.2[a](29.81)	112±9.3[a](78.12)	120±9.8[a](76.92)	112±9.5[a](78.25)
12	280±7.9[a](47.16)	56±8.6[a](89.06)	65±10.2[a](87.50)	45±7.3[a](91.26)
14	176±6.8[a](66.79)	12±2.5[a](97.65)	25±3.6[a](95.19)	18±3.2[a](96.50)
16	169±5.9[a](68.11)	00	9±2.5[a](98.26)	5±21[a](99.02)
18	164±7.2[a]	00	00	00

Results were compared with the corresponding control values (simple ointment) and p-values were calculated by Student's t-test.

[a]$p<0.001$. (Figures in the parenthesis indicate % of wound contraction.)

Table 2. Effects of extract and standard drug on incisoin wound model

Number of animals	Treatment	Tensile strength(g) (Mean±SE)
6	Simple ointment	415±12.8
6	Extract ointment (5% w/w)	546±12.2[a]
6	Extract ointment (10% w/w)	552±11.7[a]
6	Nitrofurazone ointment (0.2% w/w)	585±11.9[a]

Results were compared with control and p-value was calculated by Student's t-test. [a]$p<0.001$

strength i.e. the effect of the extract and standard drug on the wound healing process by incision wound method have been shown in Table 2. Results has been expressed as mean±SE and compared with the corresponding control (simple ointment) values, p-values were calculated by Student's t-test by comparing with control (Woodson, 1987). Percentage of wound contractions were calculated with respect to the corresponding 0 day's wound area (mm^2).

The leaf extract ointment caused significant contraction of wound in both the concentrations comparing with simple ointment (control). The results were comparable with that of standard drug nitrofurazone. The epithalisation periods of the extract ointment treated group was more than the nitrofurazone treated group of animals. In case of former it was 16±2 days where as in case of later it was 14±2 days. Tensile strength of wounds of rats treated with nitrofurazone ointment, in case of incision wound model was some what more than the extract ointment treated wounds, but all of them caused significant effect when compared with control (simple ointment) group. All these effects conclusively proved that *C. fistula* leaf extract ointment has a significant effect on healing of wounds in both excision and incision wound models in rats and thus substantiate its use in folklore medicine.

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