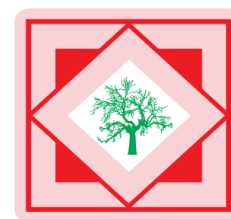




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Wound healing activity of leaves of *Cassia tora* Linn.

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ABSTRACT

The present study was aimed to evaluate the wound healing activity of extract of leaves of *Cassia tora*. Current methods used to treat wound include debridement, antibiotics, tissue graft, proteolytic enzymes and corticosteroids which posse's major drawback and unwanted side effects. Therefore the development of potent wound healer drug with fewer side effects is necessary. Wound healing activity of methanolic extract of leaves of *Cassia tora* Linn. was studied by excision and incision wound models on albino mice. Significant increase in the percentage of excision wound contraction, tensile strength of incision wound and remarkable reduction in period of complete epithelization was observed in the extract treated animals as compared to control. Granulation tissue of methanolic extract treated animals was subjected to histopathological examination showed enhance collagen formation and complete epidermis and less infiltration monocytes as compared to control. The results of present study indicated that methanolic extract ointment of *Cassia tora* Linn. exhibited significant ($p < 0.01$) wound healing activity, when compared with the control.

Key words: Excision wound model, Incision wound model, Povidone iodine

INTRODUCTION

Wound healing, or wound repair, is an intricate process in which the skin (or some other organ) repairs itself after injury [1]. The process of wound healing consists of integrated cellular and biochemical events leading to reestablishment of structural and functional integrity with regain of strength of injured tissue. Therefore the aim of treating a wound is to either shorten the time required for healing or to minimize the undesired consequences [2].

The plant *Cassia tora* Linn. (Family: *Leguminosae/Caesalpinaceae*) is a foetid annual shrub generally distributed throughout India, Srilanka, West china and tropics and it is known as Charota (Hindi), Foetid Cassia (English) [3] and Jui Mingzi (Chinese) [4]. Emodin, tricontan-1-0l, stigmaterol, beta-sitosterol-beta-D glucoside, freindlen, palmitic, stearic, succinic and d-tartaric acids, uridine, quercitrin and isoquercitrin are isolated from leaves [5]. The leaves and seeds are useful in leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis, cardiac disorders [6]. These herbs have been reported for their usefulness in the form of decoctions, infusions and tinctures in traditional system of medicines for treating skin diseases like psoriasis, leprosy etc. [7]. Antibacterial [8], hepatoprotective [9], cAMP phosphodiesterase inhibitory activity [10], antifungal [11], anti-inflammatory [12], estrogenic and antiestrogenic [13], hypolipidemic [14] and antioxidant activities [15] has been evaluated. *Cassia tora* L. leaves have anthelmintic property for the presence of flavonoid [16].

Decoctions of parts of *Cassia tora* is used as an analgesic, anticonvulsant, antipyretic, antifungal, diuretic, expectorant, laxative, purgative, treatment of glaucoma and hypertension, treatment of skin disease, ringworm and itch [17].

Review of the literature revealed that though this plant is known for several pharmacological activities, it has not been subjected to scientific evaluation for wound healing activity. Hence an attempt has been made to evaluate the wound healing property of this plant.

The leaves of *Cassia tora* were collected from different localities of Bhopal (M.P.) in the month of January and authenticated in Department of Botany, Saifia College of Science and Education, Bhopal by Dr. Zea Ul Hasan. The Reference no. is Bot. 118, 2/12/2010. A voucher specimen has been deposited in laboratory for further reference. Leaves of *Cassia tora* Linn. were dried in shade, powdered and sieved through 40-mesh size and material was stored in well-closed container.

MATERIALS AND METHODS

Preparation of extract and drug formulation

Leaves (100 gm) were extracted with methanol in soxhlet apparatus for 24 hours. The methanolic extract was then concentrated and dried. The extract was weighed and the yield obtained (14.5% w/w). The 10% formulation was prepared for topical administration, 10 g of the methanolic extract was incorporated into the 100 g of Petroleum jelly B.P. It was then filled in the wide mouth bottle container and stored in cool and dry place.

Experimental Animals

Healthy albino mice of either sex (weighing 35 to 40 gms) with no prior drug treatment were employed for wound healing activity. The animals were fed with a commercial Pellet diet (Hindustan lever, Bangalore, India) and water *ad libitum*. The animals were acclimatized to laboratory hygienic condition for 10 days before starting the experiment. The Institutional Ethical Committee (Reg. No. IAEC/ BITS-P/ 003) permitted the study.

Wound Healing Activity

Screening for wound healing activity was performed by excision wound model and incision wound model. The hair on the skin of the back surface of the animal was removed by using a suitable depilatory (Anne French hair removing cream). The selected animals were divided into three groups of six in each. Group I was assigned as control received simple ointment base I.P. Group II received the standard drug (5% w/w povidone-iodine ointment). In an identical manner. Group III received 10% w/w methanolic extract ointment of leaves of *Cassia tora*.

Excision Wound Model

A circular wound of about 10 mm diameter was inflicted on depilated dorsal thoracic region of the mice under topical anaesthesia with 4% xylocane. Drugs were applied once daily and measurement of wound area was done by using vernier calipers on 4th, 8th and 12th post wounding day. The wound closure was measured at regular interval of time to see the percentage of wound closure and epithelization time that indicate the formation of new epithelial tissue to cover the wound. Wound contraction was calculated as percentage reduction in wound area (mm²). The evaluated surface area was then employed to calculate the percentage of wound contraction, taking the initial size of the wound, 100 mm², as 100%, by using the following equation [18].

$$\text{Percentage of Wound contraction} = \frac{\text{Initial wound size} - \text{specific day wound size}}{\text{Initial wound size}} \times 100$$

Incision Wound Model

In the incision model [19] all mice were anaesthetized before wound creation by mild ether anesthesia. Paravertibral long incision were made through the skin at a length of about 1.5 cm from the midline on the depilated back of mice with the help of sharp blade. The incision was sutured using 4-0 silk thread with the help of a straight rounded bodied needle. Suture were removed on the 8th post wounding day and the tensile strength was determined on the 10th post wounding day by continuous constant water flow technique [20].

Histopathological studies

In the histopathological studies, newly formed skin of control, standard and treated group were excised together with the surrounding skin on the 12th post wounding day by fixing the skin in 10% buffered neutral formalin for 24 hour

and dehydrated with a sequence of ethanol- xylene series of solution[21]. The materials were infiltrated and embedded with paraffin (40-60 °C). Microtome sections were taken at 10 μ thickness. The sections were processed in alcohol-xylene series and stained with hemotoxylin-eosin dye. The histological changes were observed under a microscope. Microscopic photographs of collagen tissue were taken as were shown in the figure (1, 2, 3).

Statistical analysis

The results of these experiments are expressed as mean \pm SD. All the results were analyzed statistically using Student's t-test to identify the differences between the treated and control. The data were considered significant at $p < 0.01$.

Table 1: Percentage wound Contraction Area on the following Post Wounding Days

S.no	Drug	Percentage wound contraction area (mm ²) on a days (mean \pm SD)		
		4 th day	8 th day	12 th day
1.	Control (simple ointment base I.P.)	31.13 \pm 1.32	63.1 \pm 0.35	73.22 \pm 0.788*
2.	Standard (povidone iodine)	72.72 \pm .68	89.39 \pm 0.72	99.9 \pm 0.02
3.	Treated (10% methanolic extract)	52.54 \pm 0.46	85.08 \pm 1.56	99.07 \pm 0.622*

Each value is a mean percentage closure area \pm Standard Deviation for group of six animals (n=6). Since there is significant difference in the control and the treated group values. *Indicates statistically significant difference at $p < 0.01$ when compared to control group.

Table 2: Effect of Methanolic Extract Ointment of *Cassia tora* Linn. on Incision Wound Model

S.no.	Drug	Tensile strength in gm/cm on 10 th post wounding day (Mean \pm SD, n=6)
1.	Control	174.93 \pm 0.875
2.	Standard	416.4 \pm 0.947
3.	Treated	390.86 \pm 0.938

Each value are in mean \pm standard deviation for the group of six animal (n=6). Statistically significant difference in comparison with the control group. $p < 0.01$

RESULTS AND DISCUSSION

In excision wound model, percentage closure of wound area of the test formulation treated animals were increased significantly ($p < 0.01$) on 4th, 8th and 12th post wounding days in comparison with control group of animals (Table 1). The data reveals that the rate of wound contraction was significantly higher in the animals treated with the methanol extract ointment and it is comparable with standard drug (povidone iodine).

In incision wound model, methanolic extract ointment showed significant increase in the tensile strength compared to the control. Maximum tensile strength were seen in groups treated with standard (416.4 \pm 0.947g) and methanolic extract (390.86 \pm 0.938 g) which were statistically significant ($p < 0.01$) from control group (174.93 \pm 0.875 g); suggest that the extract promoted wound healing activity. (Table 2).

In the histopathological study of granulation tissue of methanolic extract treated animals showed significant increase in the collagen deposition, complete epidermis and less infiltration monocytes compared to control.

CONCLUSION

In the present study, phytochemical investigation of leaves extract of *Cassia tora* Linn. showed the presence of flavonoids, tannins and phenolic compound, glycosides and steroids. Several phytoconstituent like tannin [22] and flavonoid [23] compounds are known to promote the wound-healing process mainly due to their antioxidant and antimicrobial activities, which seems to be responsible for wound contraction and increased rate of epithelialisation. Lipid peroxidation is an important process in several type of injury like burn, infected wound and skin ulcer etc. Some of the phytoconstituents like flavonoids are known to reduce lipid peroxidation not only by preventing or slowing the onset of cell necrosis but also by improving vascularity. Hence any drug that inhibits lipid peroxidation is believed to increase the viability of collagen fibrils by increasing the strength of collagen fibres results in increasing the circulation, preventing the cell damage and promoting the DNA synthesis and slowing the rate of necrosis [24]. Thus the present study revealed that the methanolic extract of *Cassia tora* leaves possesses significant wound-healing activity which may be attributed to the phytoconstituents present in it, which may be either due to their individual or additive effect that fastens the process of wound healing. Hence the present investigation offer scientific evidence to the traditional use of *Cassia tora* leaves.



Figure 1. shows less collagen formation and incomplete epidermis in the control group. H & E Stain, Magnification 10x

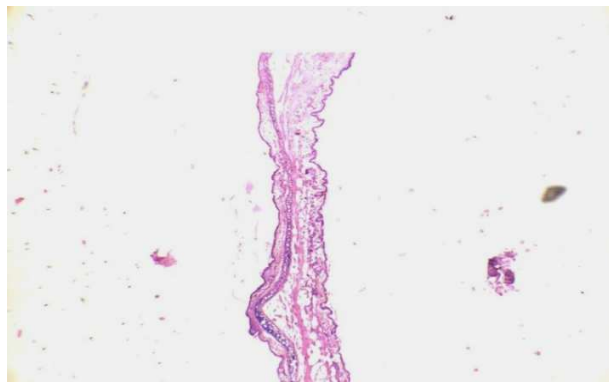


Figure 2. shows Normal morphology & less infiltration monocytes which received the treatment of Standard. H & E Stain, Magnification 4x

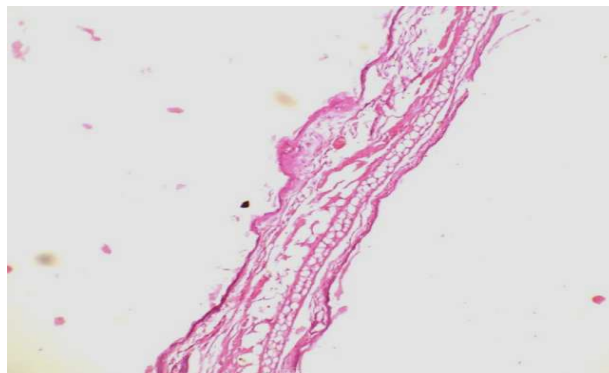


Figure 3. shows enhance collagen formation and complete Epidermis and less infiltration monocytes which received the treatment of *Cassia tora* extract ointment. H & E Stain, Magnification 10x

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