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Formulation and evaluation of Wound Healing activity of methanolic leaf extract of *Cassia tora*

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ABSTRACT

Formulation and evaluation of wound healing activity of methanolic leaf extract of ***Cassia tora***. The extract of ***Cassia tora*** (300mg/kg/day) applied topically, was evaluated for its wound healing activity in albino wistar rats (150 - 200gm body weight) using Excision and Incision wound models for 16days and 10days respectively. Wound healing activity was studied by determining wound breaking strength (g), percentage of wound contraction and period of epithelization. Methanolic extract treated animals exhibit 98.07% wound contraction when compared to control which was 90.54%. Extract treated wounds are found to epithelize faster as compared to control. Significant ($P < 0.05$) increase in the wound breaking strength (280.31 ± 6.521) was observed. 5% Betadine ointment was used as a standard. The ***Cassia tora*** methanolic leaf extract showed significant wound healing activity when topically administered on rats thus, supporting this traditional use.

Key words: Betadine ointment; Excision wound; Incision wound; Period of epithelization.

INTRODUCTION

Medicinal plants have been used since time immemorial for treatment of various ailments of skin and dermatological disorders especially cuts, wounds and burns (Govindrajan *et al.* 2007). Wound is defined as a loss or breaking of cellular and anatomic or functional continuity of living tissues. Wound healing is the natural process of body for regenerating dermal and epidermal tissue (Deodhar *et al*. 1997), it involves continuous cell-cell and cell-matrix interactions that allow the process to proceed in three overlapping phases viz. inflammation, cellular proliferation and remodeling, respectively. Inflammation phase of 0-3 days which involves migration of neutrophils around incision. Proliferation is of 3-12 days in which incisional space is

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filled with granulation tissue. Remodeling phase is of 3-6 months which involves synthesis of collagen fibers leading to increase in tensile strength of the skin (Cotran *et al.* 1997).

MATERIALS AND METHODS

Plant Materials- The leaves of ***Cassia tora*** were collected from local surroundings of Vidisha (M.P.) and kept it to dryness in shady area. The plant was identified and authenticated by Dr. S.K.Jain. Department of Botany S.S.L. Jain P.G. College Vidisha M.P. India, and voucher specimen no. Ca/t/00810.

Preparation of leaves extract- The extract was prepared from the plant material for the wound healing activity testing. Initially, the powdered plant material was kept in n-hexane for removing the fatty material of the plants then, extract (Yield; 11.89%) was obtained from the dried powdered leaves (750 gm) of *Cassia tora* using 100% methanol by cold percolation method as described in folk medicine for the assessment of wound healing activity. Then evaporated to dryness to give semi solid crude using vacuum evaporator. The dried extract was stored at 2-8 °C in refrigerator. The extract was further used for the evaluation of wound healing activity.

Animals- The wistar albino rats of either sex, weighing 150-200g were housed under standard environmental conditions of temperature and humidity (25±0.5°C) and 12 h light/ dark cycle were utilized for the studies. The animals were fed with standard pellet diet and waster ad *libitum*. The animal studies were performed in Pest Control and Ayurvedic Drug Research Laboratory, S.S.L.Jain P.G. College Vidisha with permission from Institutional animal ethical committee with India .CPCSEA No .407/03/CA/CPCSEA.

Acute toxicity study- Acute toxicity study was performed according to OECD guidelines No. 423 (OECD, 2000). Mice were kept overnight fasting prior to drug administration. Swiss albino mice of either sex were divided into two groups with six animals each, one control group and other group received a single oral dose (2000 mg/kg, body weight) of methanolic extract of leaves of ***Cassia tora*** (Linn.) After the administration of *Cassia tora* methanol extract food was withheld for further 3–4 h. Animals were observed individually at least once during the first 30 min after dosing, periodically during the first 24 h (with special attention during the first 4 h) and daily thereafter for a period of 14 days for the symptoms of toxicity and death.

Acute dermal toxicity- The study was carried out to determine the therapeutic dose of methanolic extracts. The acute dermal toxicity testing of methanolic extract was done by applying the ointment containing methanolic extract of the highest concentrations of 2 % (w/w) on the shaved back of the rats. The OECD guidelines No. 402 (OECD, guidelines, 1987) were followed for the study.

Wound healing activity- The animals were grouped into 3 groups (6 animals in each group) viz. control, standard and one group for testing methanolic extract. The control group was treated with simple ointment base B.P. The standard group was treated with Betadine 5% (/weight/ weight) povidone iodine ointment. The test group was treated with ointments with highest concentration 2 % (weight / weight) of extract incorporated in simple ointment base, in all the models.

Excision wound model- The rats were anesthetized by administering ketamine (0.5 ml/kg body weight intra peritoneal). A full thickness of excision wound of circular area (approx. 500 mm2 and 2 mm depth) was made on the shaved back of the rats 30 min later the

administration of ketamine injection. The wounding day was considered as day 0 (Fig.1). The wounds were treated with topical application of the ointments as described above till the wounds were completely healed.

Incision wound model- The rats were anesthetized by administering ketamine (0.5 ml/kg b.w.i.p.). Incision wounds of about 6 cm in length and 2 mm in depth were created with sterile scalpel on the shaved back of the rats 30 min later the administration of ketamine injection. The parted skin was kept together and stitched with black silk at 0.5 cm intervals Surgical thread (No. 000) and a curved needle (No. 9) were used for stitching. The continuous thread on both wounds edges were tightened for good closure of the wounds. The wounds of animals in the different groups were treated with the topical application of the ointments as described above, for the period of 10 days. The wounding day was considered as day 0. (Fig. 4). When wounds were cured thoroughly, the sutures were removed on the 8th post wounding day of Test and Standard (Fig.5 & Fig.6) and the tensile strength of the skin that is the weight in grams required to break open the wound/skin was measured by tensiometer on the 10th day reported in Table 2 (Nath *et al.* 2006). Tensile strength was calculated using the following formula (Diwan *et al.* 2008):

$$\text{Tensile Strength} = \frac{\text{Breaking Strength (g)}}{\text{Cross- sectional area of skin (mm2)}}$$

Statistical analysis- Results obtained from the four wound models have been expressed as mean ± SEM and were compared with the corresponding control group (simple ointment B.P.) by applying ANOVA test (Mukherjee *et al.* 2000).

$$\% \text{ of wound closure} = \frac{\text{Wound area on day 0 –day n x 100}}{\text{Wound area on day 0}}$$

RESULTS AND DISCUSSION

Excision wound study- The results of wound healing activity by excision wound model are presented in Table (1) and Fig. (3). The value presented in the table represent percentage wound healing at 4, 8, 12, 16 days for control (simple ointment B.P. treated group), standard (povidone iodine treated group) and the test group viz. methanolic extract (2% w/w). It is noticed that wound healing power of rats treated with ointment containing 2 % (w/w) methanolic extract was found to be significantly higher ($P<0.001$) on day 16th as compared to control group. The epithelization period was also found to be the least that is 21 days in case of animals treated with ointment containing 2% (w/w) methanolic extract.

Fig 1: The excision wounds were monitored and the area of wound was measured at day 0 and day 16 (test and standard)

Table 1: Effect of topical application of ointment containing methanolic extract of *Cassia tora* leaves on wound healing contraction of excision wound in rats.

Groups	4 day	8 day	12 day	16 day	Period of epithelization (days)
Control	24.13±2.2	69.63±1.7	88.34±1.5	90.54±0.6*	25th day
Standard	37.81±2.6	77.56±1.5	94.85±0.5*	99.60±0.2***	18th day
*C.T.*Meth.Extra.	32.33±2.4	73.24±1.8	91.62±0.8**	98.07±0.4***	21th day

Incision wound study- The results of wound healing activity by incision wound model are presented in Table (2), Fig. (4). This study was evaluated by measuring the tensile strength of the incision wound of

different groups viz. control treated with simple ointment base B.P., standard group treated with drug povidone iodine and the test group treated with highest concentration of methanolic extract. The results are presented as mean weight in gram ± SEM. The rats treated with ointment containing 2% (w/w) methanolic extract indicated significantly strength (35.90 ± 2.43) as compared to control group (16.14 ± 2.84).

Fig. 2: Theincision wounds were monitored and the area of wound was measured at day 0 and day 8 (test and standard)

Table 2: Effect of topical application of ointment containing methanolic extract of *Cassia tora* leaves on tensile strength of the skin having incision wound in rats.

Groups	Tensile strength (gm)
Control	16.14±2.84
Standard	47.47±2.56***
C.T. meth. Extract	280.09±2.43**

The present study, it was aimed to minimize tissue damage and provide an adequate tissue perfusion and oxygenation, proper nutrition and moist wound healing environment to restore the anatomical continuity and function of the affected part. In the present study, rats were treated with *Cassia tora* methanolic **e**xtract 2% w/w ointment for 16 days period taking observation in every 4th day, it was noticed that complete epithelization of both the wound models take about 16 days. This is the period of complete healing of wound.

Thus, it can be conclude that the plant extract at 300 mg dose/kg body weight can be a good solution for the healing of both the wounds. Thus, the folklore claim for the use of leaves in healing of the *Cassia tora* wounds can be justified by the present study.

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