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Research Article

Evaluation of *In Vivo* Wound Healing Activity of Cassia Tora Leaf Extract

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ABSTRACT
Plant Cassia tora also known as Chakra Marda or Puvad was found in most part of India and it was previously used in treatment of common skin ailments by Indian tribes. However, there are no scientific evidences justifying use of Cassia tora in wound healing. Therefore, the present study was performed to evaluate wound healing activity of Cassia tora leaf methanolic extract. These activities were studied by two animal models i.e. Excision wound model and incision wound model in Wistar rats. In excision wound model compared to the control group, percentage contraction was significantly higher in Cassia tora (2%) treated group. Period of epithelization was also found to be significant in treatment group as compared to control group. In incision wound model, tensile strength of the wound was measured. Statistical significance(p) calculated by ANOVA followed by Dunnet's test reveals that Cassia tora extracts shows significant wound healing activity in Wistar rats.

Keywords: Cassia tora, wound healing, Incision wound, Excision wound, Animal models.

INTRODUCTION

The integumentary system is the largest system of the body. It makes up approximately 16% of body weight, is 1.5 - 2 square meters in area and consists of the largest organ of the body, the skin, and the accessory structures: sebaceous and sudoriferous glands, hair and nails[1,2]. Wounds are inevitable events in everyday life that may occur due to physical, chemical, thermal, microbial or an immunological insult to the tissue. The wound is determined as a disruption of cellular and anatomical continuity of the tissue[3]. A combination of cellular and biochemical events leading wound healing and re-building of the injured tissue strength and integrity. Clinically, one often faces under healing, over healing and non-healing. Therefore, it is intended to treat the wound to reduce the time required for healing or considered to reduce the undesirable effects. These wound healing can be enhanced by complete asepsis, removal of devitalized tissue, apposition of wound edge and regular dressing. In addition, certain herbs which possesses antiseptic, astringent, anti-microbial and bio-stimulatory property can also enhance the rate of healing[4].

Plant material *Cassia tora* is a small shrub with a wide geographical distribution as a weed in almost all the Asian countries. Plant belongs to Family Leguminosae that is also known as Pea or bean family that is large and having economic importance in flowering plants[5]. *Cassia tora* is a wild collect that grows in most of the regions of India as a weed. The main useful parts of *Cassia tora* are leaves, roots and seeds. Cassia tora has been stated to contain many active constituents, including Anthraquinones, emodin, rhein, quercetin, chrysophenol etc[6].

METHODOLOGY

Collection and identification of plant material

The plant material was collected after a few months of rain in the month of September and October from Surendranagar district. It was identified by department of Pharmacognosy, C. U. Shah University and herbarium specimen was stored. Dirt particles were removed and it was subjected for shade drying for 15-20 days. After drying, leaves were grinded in a lab scale grinder. A fine powdered is prepared and it is stored in an air tight light resistant container for further use.

Preparation of Plant extract[7,8]

The fresh leaves of *Cassia tora* was shade dried and powdered. The powder was extracted successively with Petroleum ether (60:80), Chloroform and Methanol using Sohxlet Apparatus. After the Soxhlet extraction, a thick dark solvent with extracted material is accumulated in the round bottom flask. Extra solvent was removed by suction pump. Extract was collected and dried in a porcelain dish and placed into desiccator. This extract was further used for experimental purpose.

Pharmacological Activity

There can be various skin problems starting from common itching and rashes. On continuous itching and rashes, condition may become worst and development of dermatitis or wound like lesion occurs. Therefore, these activities needed to be evaluated.

Animals used in Study

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Table 1: Wound healing area (mm^2) with days (Day 0-15).

Day	0	3	6	9	12	15
Control	497.67±2.08	458.00±5.29	389.67±4.51	306.00±4.00	265.33±4.16	205.33±4.16
Std.	510.00±2.00	349.67±5.03	214.67±6.51	127.33±4.16	42.67±4.16	7.33±1.53
Test	513.33±2.89	362.00±2.00	231.00±4.58	117.33±3.06	51.33±3.06	11.00±1.73

Values are expressed as Mean ± S.D.; Statistical significance (p) calculated by ANOVA followed by Dunnet's test $p<0.05$ calculated by comparing treated group with control.

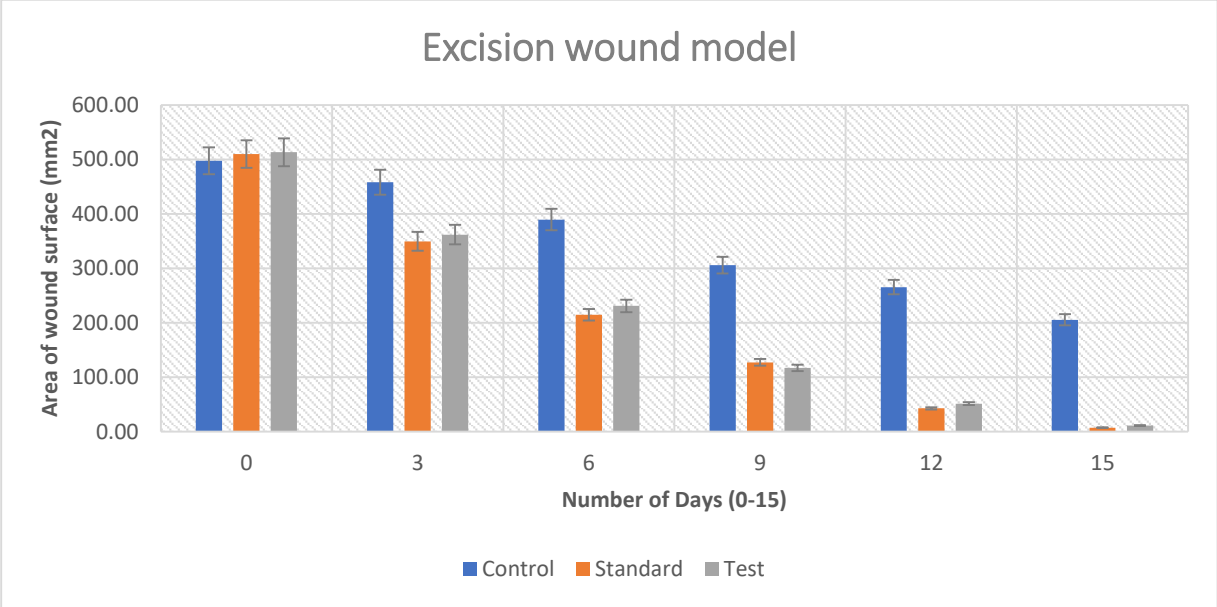

Chart 1: Excision Wound Model.

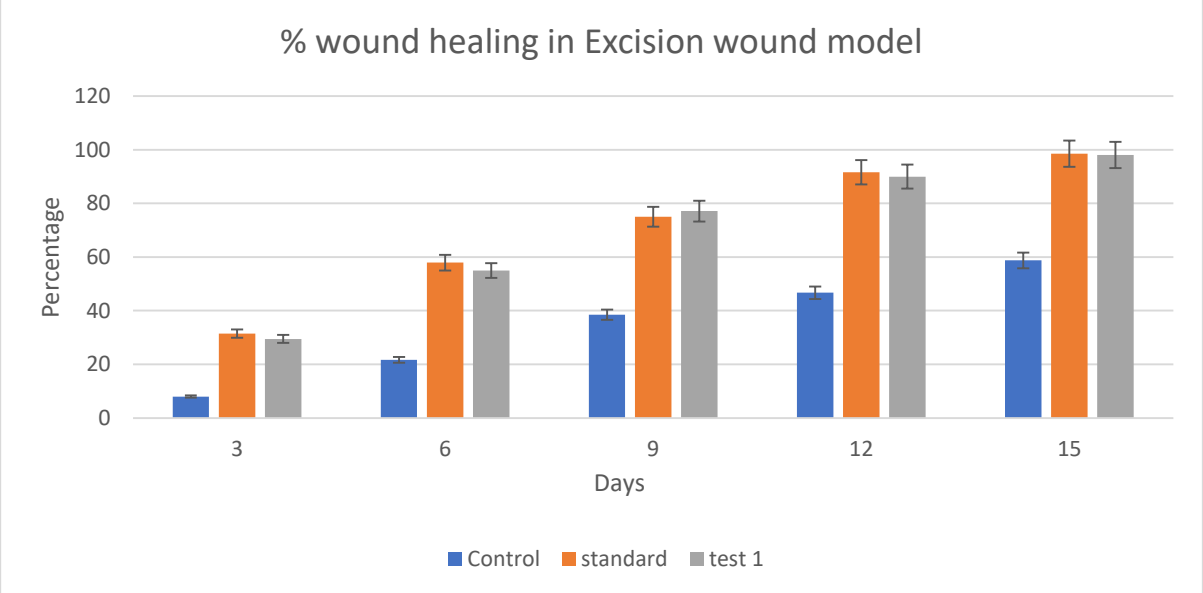

Chart 2: Graph indicating % wound healing.

For experiments on animal models, male wistar albino rats were selected in which each rat weighs about 150-150gm. After one week of acclimatization to environment of laboratory, rats were used. They were kept in the animal house of the department of pharmacology in light dark cycles of 10 and 14 h at 26±2 ◦C, respectively. Animals were given rodent diet and water *ad libitum*. All the investigational procedures were permitted by Institutional animal ethical committee Approved protocol no. IAEC/CTIPS/2015/11/0011(PCE-U)

Grouping of Animals

Control (4)

Standard (4)

Test (2% CTE) (4)

Excision wound model[4,9]

Wistar rats in each group were anaesthetized by anesthetic ether. The back portion of rat was. Rat skin was excised in

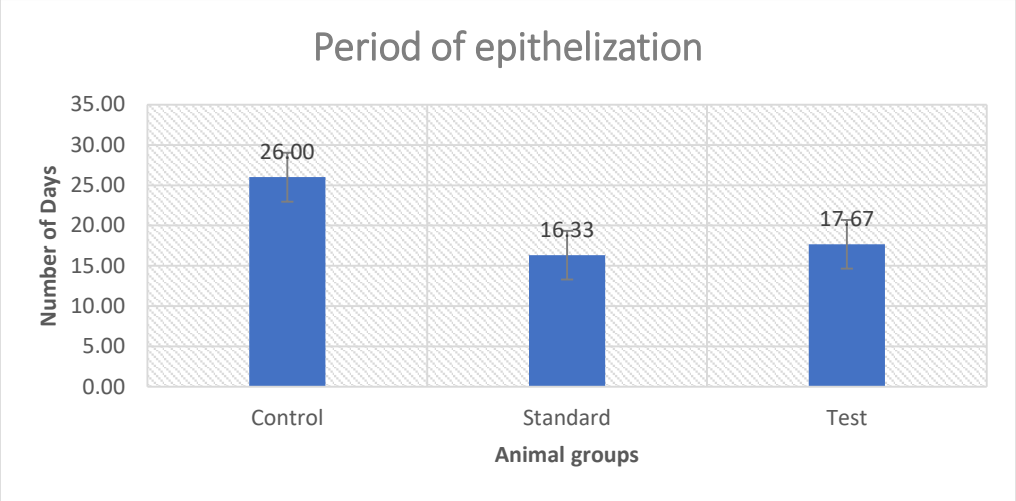

Chart 3: Graph indicating Period of Epithelization.

Table 2: Period of Epithelization.

Excision wound	Period of epithelization (in Days)
Control	26.00±1.00
Standard	16.33±0.58
Test	17.67±0.58

Tensile strength by incision wound model.

Sr. No	Group	10th Past wound day Tensile Strength
1	Control	160.7±4.16
2	Standard	90.5±5.29
3	*Cassia tora* extract treated	120±3.06

predetermined 500 mm^2 area of full thickness and wound was created. It was left undressed to open environment. For the period of 15 days, extracts and standard were administered on wounded surface. During treatment, contraction of wounds which leads to healing were studied by tracing raw surface of affected part. Area of wound was measured on milli-meter scale graph on every alternated day and degree of wound healing was calculated.

From the reduction in wound area, Contraction of wound was calculated by following formula:

$$\% \text{ of wound closure} = \frac{\text{Area of wound on day 0} - \text{area of wound on day N}}{\text{Area of wound on day 0}} \times 100$$

Where N = number of days 3rd, 6th, 9th, 12th and 15th day

Period of epithelization was also calculated and compared with that of control group.

Incision wound model[10,11]

Para vertebral straight incision of 6 cm length was made through the entire thickness of the skin, on either side of the vertebral column with the help of a sharp scalpel. After complete hemostasis, the wounds were closed by means of interrupted sutures placed at approximately 1 cm apart. Animals were treated daily with drugs, as mentioned above under excision wound model from 0 days to 9th post wounding day. The wound breaking strength was estimated at 10 the day by tensile tester. Tensile strength was calculated using the following formula:

Tensile strength = Total breaking load / Cross-sectional area

RESULTS AND DISCUSSION

Wound Healing Activity

In vivo wound healing activity was carried out on Wistar Rats as per procedure discussed above. Results of Excision wound model are shown in Table 1.

Period of Epithelization

Period of epithelization is defined as days required to grow epithelial tissue in wound area of excised animals. In current study, period of epithelization was shown in table below:

CONCLUSION

Wound healing activity of Cassia tora leaf methanolic extract was studied by Incision and excision wound model using Wistar Rats. Various parameters like wound area, % wound closure, period of epithelization and tensile strength was studied. *Cassia tora* extract (2%) shows significant wound healing activity as compared to control group. Thus, it may emerge as an economic and safer alternative as wound healing agent.

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