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Evaluation of aqueous extract of Roots of *Carica papaya* on wound healing activity in albino Rats

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ABSTRACT

Wound is a general complication seen all over the world and plants are mostly considered as a primary remedy to heal wounds. Lot of research on medicinal plants is now on par with synthetic molecules for decades to heal wounds, though a smaller complication yet dangerous if left untreated. Traditionally plant materials are used to treat and heal wounds for several thousand years. All the Indian systems of medicine, Ayurveda, Siddha and Unani describe drugs of plant, mineral and animal origin to treat and heal wounds. Plants are given prime importance as they are easily available but scientific evidence for their wound healing activity is available for only few plant materials. The present study was aimed to evaluate the wound healing potential of aqueous extract of roots of carica papaya. It is a well known plant in Indian traditional medicine. On the basis of traditional use and literature references, this plant was selected for evaluation of wound healing potential. An aqueous extract of root was examined for wound healing activity at doses 200 mg/kg which was evaluated for its wound healing activity in albino rats using excision and incision wound models. Latex treated animals exhibit 89.40 % reduction in wound area when compared to controls which was 80.38 %. The extract treated wounds are found to epithelize faster as compared to controls. Significant ($p<0.001$) increase in granuloma breaking strength (578.99±0.96) was observed. The Framycetin sulphate cream (FSC) 1 % w/w was used as standard.

Keywords: *Carica papaya*, Wound healing, Excision wound, Incision wound, Framycetin sulphate cream.

INTRODUCTION

A wound is a disruption in the continuity of cells anything that causes cells that would normally be connected to become separated. Wound healing is the restoration of that continuity. Several effects may result with the occurrence of a wound: immediate loss of all or part of organ functioning, sympathetic stress response, hemorrhage and blood clotting, bacterial contamination, and death of cells. Herbal remedies are considered the oldest forms of health care known to mankind on this earth. The parts of the plant used for medicinal purposes are leaves, root, stem, fruits, the complete aerial parts, the whole plant, barks (root and stem) and flowers. *Carica papaya Linnaeus*, (pawpaw), belongs to the family of Caricaceae. Papaya is not a tree but an herbaceous succulent plants that posses self supporting stems [1]. Papaya is a large perennial herb with a rapid growth rate. The plants are usually short lived, but can produce fruit for more than 20years. The papaya has a rather complicated means of reproduction. The plants are male, hermaphrodite, or female [2].The male trees are uncommon, but sometimes occur when homeowners collect their own seeds. Hermaphrodite trees (flowers with male and female parts) are the commercial standard, producing a pear shaped fruit. These plants are self pollinated [3]. *Carica papaya* plants produce natural compounds (annonaceous acetogenins) in leaf bark and twig tissues that possess both highly anti-tumour and pesticidal properties. It was suggested that a potentially lucrative industry based simply on production of plant biomass could develop for production of anti-cancer drugs, pending Food and Drug Agency approval, and natural (botanical) pesticides [4]. The high level of natural self-defence compounds in the tree makes it highly resistant to insect and disease infestation [5].

EXPERIMENTAL SECTION

Plant source:
Healthy disease free, mature fresh plant root sample were collected locally from Bhopal (M.P.), India. Fresh root were washed thoroughly 2-3 times with running tap water and once with sterile water, shade-dried without any contamination. The dried leaves were then powdered using an grinder.

Animals:
Swiss albino rats were obtained from animal house of **Pinnacle Biomedical Research Institute Bhopal, Madhyapradesh, India**. The experiment was conducted as per the permission of Institutional animal ethical committee (IAEC) of PBRI (Regd. No. 1283/c/09/CPCSEA). All conditions were maintained according to CPCSEA norms. The animals of either sex were selected randomly of uniform weight 120±5 gm from animal house. The room temperature was maintained 22±2°C with food (Lipton India Ltd. pellets) and water *ad libitum*. The animals were transferred to the laboratory at least 1h before the start of the experiment. The experiments were performed during day (08:00-16:00 h). The institutional animal ethical committee approved to the study protocol**.**

Acute dermal toxicity – fixed dose procedure:
The acute dermal toxicity study was carried out in adult female albino rats by “fix dose” method of OECD (Organization for Economic Co-operation and Development) Guideline No.434. Latex of the plant *carica papaya* was applied topically at dose level 2000 mg/kg.

Selection of dose:
For the assessment of cutaneous wound healing activity, dose level was chosen in such a way that, dose was approximately one tenth of the maximum dose during acute toxicity studies (200 mg/kg/day).

Grouping of animals:
Animals were divided in to three groups, each group consisting of 6 rats.

Group I: *Received no treatment and served as control*
Group II: *Received application of standard drug ointment i.e. Framycetin sulphate cream (FSC) _ (1 %w/w)*
Group III: *Received application of latex of carica papaya (200 mg/kg/day)*

Wound Healing Activity
Excision wound model:
Excision wounds were used for the study of rate of contraction of wound and epithelization. Animals were anaesthetized with slight vapour inhalation of di-ethyl ether and the right side of each rat was shaved. Excision wounds sized 300 mm2 and 2 mm depth were made by cutting out layer of skin from the shaven area. The entire wound was left open. The treatment was done topically in all the cases. The latex was applied at a dose of 200 mg/kg/day for 16 days. Wound areas were measured on days 1, 4, 8 and 16 for all groups, using a transparency sheet and a permanent marker [6].

Incision wound model:
The incision wound model was studied. Under light ether anesthesia the animal was secured to operation table in its natural position. One paravertebral straight incision of 6 cm was made on either side of the vertebral column with the help of scalpel blade. Wounds were cleaned with 70% alcohol soaked with cotton swabs. They were kept in separate cages. The latex was applied at a dose of 200 mg/kg/day for 10 days. The sutures were removed after 8 days, on tenth day the tensile strength was measured by continuous constant water supply technique [7-8].

Statistical analysis:
The mean±S.E.M. statistical significance of difference of control and test data was determined by ANOVA. Simple oneway analyses of variance were used for different doses within a group. A probability value of 0.05 or less was taken to indicate statistical significance.

RESULTS AND DISCUSSION

In both the models studied, significantly improved wound-healing activity has been observed with the *carica papaya* root extract, compared to that of the reference standard and control group of animals. During study of wound healing in normal rats following results were obtained: Acute toxicity studies showed that drug was found to be safe up to maximum dose of 2g/Kg body weight of the animal. In studies using excision wound model, the latex treated group III showed significantly greater wound healing as compared to control animals (Table 1).

Table 1. Effect of Latex of *carica papaya* on Excision Wound [Wound Area (mm2)

Day	Group I	Group II	Group III
0	315.2±0.91	266.6±0.44	197.8±0.63
4	294.6±0.18	187.7±0.76	148.8±0.60
8	237.6±0.001	127.4±0.07	109.54±0.01
16	88.5±0.58	39.09±0.58	43.9±0.62

n=6; values are in mean ±SEM, Significant p<0.00

Table 2: Effect of latex of *carica papaya* on excision wound (% wound closure)

Percentage Wound Closure = (Initial area of Wound – NTH day area of wound) x 100/ (Initial area of Wound)

Day	Group I	Group II	Group III
0	**0**	**0**	**0**
4	17.44%	25.43%	21.56%
8	39.56%	41.55%	40.44%
16	80.38%	94.55%	89.40%

Table 3: Effect of latex of *carica papaya* on wound healing in incision wound

Groups	Incision wound breaking strength (g)
Group I	366.3±0.07
Group II	520.11±0.94*
Group III	578.99±0.96***

*n=6; values are in mean ±SEM, ***Very Significant p<0.0001, *Significant p<0.05*

Wound healing is a process by which a damaged tissue is restored as closely as possible to its normal state and wound contraction is the process of shrinkage of area of the wound. It mainly depends on the repairing ability of the tissue, type and extent of damage and general state of the health of the tissue. The granulation tissue of the wound is primarily composed of fibroblast, collagen, edema, and small new blood vessels. The undifferentiated mesenchymal cells of the wound margin modulate themselves into fibroblast, which start migrating into the wound gap along with the fibrin strands. In both the models studied, significantly improved wound-healing activity has been observed with the *carica papaya* root extract, compared to that of the reference standard and control group of animals. In present study incision wounds healing by granulation, collagenation, and tensile strength was measured indirectly to assess the collagen content and maturation. The results indicate that latex of *carica papaya* root extract significantly promoted collagen as compared to that of control. Use of single model is inadequate and there is no reference standard which can collectively represent the various components of wound healing as drugs which, influence one phase may not necessarily influence another. Hence in our study we have used two models to assess the effect of latex on various phases of wound healing.

CONCLUSION

The wound healing activity of latex of *carica papaya* root extract was studied by using excision and incision wound model and the latex showed the significant wound healing activity as like as

standard FSC (Framycetin sulphate cream) and it may be suggested for treating various types' wounds in human beings. Further studies with purified constituents are needed to understand the complete mechanism of wound healing activity of *carica papaya*.

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