ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Evaluation of Wound-Healing Activity on the Bark Extract of *Carica papaya* Linn. In Albino Rats.

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ABSTRACT

Traditionally, *Carica papaya* is used to dispose of diverse skin-disorders, including wounds. The previous studies in *Carica papaya* bark suggests that it contains components like β-sitosterol which are known to promote epithelization. So the recent study was aimed at investigating the healing efficiency of Methanolic bark extract of *Carica papaya* Linn. Albino rats were divided into four groups. Group-1(control) receives simple ointment base, Group-2 (standard) receives povidone-iodine ointment, Group-3 (Low-dose) and Group-4 (high-dose) recieves 200mg/kg b.w. and 500 mg/kg b.w. of Methanolic extract of Carica papaya Linn. bark mixed with ointment base respectively. These groups were studied for its effect on wound healing using incision, excision and dead space-wound models. The plant bark extract showed an explicit, positive effect on wound healing, with decrease in period of epithelization, increase in the rate of wound contraction, skin breaking strength, granulation tissue dry weight content and breaking strength of granulation tissue. It also showed that there is a significant elevation in the levels of antioxidant enzymes, Superoxide dismutase and catalase, in the granuloma tissue. The efficacy of the extract may be because of its action on antioxidant enzymes.

Key words: Wound healing, hydroxyprolin, superoxide dismutase, catalase, *Carica papaya*,

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ISSN: 0975-8585

INTRODUCTION

In the regular tissue continuum wound is a breach, which results in a variety of cellular and molecular sequelae. The basic principles of optimal wound healing which include minimizing tissue damage, debriding nonviable tissue, maximizing tissue perfusion and oxygenation, proper nutrition and moist wound healing environment have been recognized for many years [1]. Starting from simple non-expensive analgesics to complex and expensive chemotherapeutic agents administered in the management of wound, a number of drugs affect healing either positively or negatively [2]. Wounds are unavoidable dealings of life which take place due to bodily injury, chemical injury or microbial infections. Healing of wounds generally takes place in a route away from its ordinary course. Normally, under healing, over healing or no healing of wounds is common. Management of under healing wounds is a complex and expensive program and in modern biomedical sciences research on drugs that enhance wound healing is a developing area. Numerous drugs obtained from plant origin are known to enhance the healing of unusual types of wounds. Despite the fact, that a few of these drugs have been screened scientifically for evaluation of wound healing activity in different pharmacological models and patients, the potential of several of the traditionally used herbal agents reside behind unexplored. Only in a small number of cases recognition of the active chemical constituents was possible [3].

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 [4]. Its extract showed wound healing property after its topical application in straptozotocine induced diabetes mellitus rats[5]. The wound healing activity of latex of C*arica 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 Framycetin sulphate cream [6]. Another interesting investigation showed that aqueous extract of *Carica papaya* leaves had wound-healing potential on rats. The *Carica papaya* Linn. Bark has β-Sitosterol, glucose, fructose, sucrose, galactose and xylitol[7,8,9]. β-Sitosterol a constituent of *Carica papaya* bark promotes epithelialization[10]. Since a little information is available about the wound healing potential of *Carica papaya* stem bark, it was considered worthwhile to study the wound healing potential of methanolic stem bark extract on albino rats.

MATERIALS AND METHODS

Plant Material

The stem bark of *Carica papaya* was collected during June 2013 from Kalabari, Sonitpur district, Assam state, India. The sample was authenticated in the research and development laboratory of Natural Remedies pvt. Ltd. Bangalore of Karnataka State, India, by comparing the sample with authentic sample. A voucher specimen with Batch Number CP/391 has been preserved at the Laboratory for further reference.

Preparation of Plant Extracts

The bark was dried in the shed and coarsely powdered, which was extracted with methanol in a soxhlet apparatus for 72h. The methanolic extract obtained was concentrated

in a rotary evaporator at a reduced pressure. The concentrated extract was finally evaporated to form a dried mass, which was subjected to various chemical tests in order to detect the presence of different chemical constituents [11].

Preliminary Phytochemical Analysis

The methanolic extract isolated from *Carica papaya* Linn. Bark (MCPB) was screened for the presence of various phytoconstituents according to the phytochemical methods described by Harborne[12].

Experimental Animals

Adult Albino rats (150 to 200 gm) were used for all the experiments in the present study. The animals were maintained under standard husbandry conditions in the animal house of 'The oxford college of Pharmacy' (temperature 25 ± 2°C) in a natural light-dark cycle and fed with standard rodent diet and water ad libitum. Ethical committee clearance was obtained from IAE (Institutional Animal Ethics Committee) of CPCSEA (Ref. No.461/01/C/CPCSEA)

Acute toxicity studies

The acute toxicity of MCPB was determined as per the OECD guideline no. 423 (Acute toxic class method)[13]. It was observed that the rats were not mortal even at 2000 mg kg-1 dose of MCPB. Hence, 1/5th (500mg/kg) and 1/10th (200 mg/ kg) of MCPB were selected as high dose and low dose respectively for this study.

Excision wound model

The infliction of rats with excision wounds was done according to the method described by Morton and Malon[14]. For excision wound, a 500 mm2 full thickness of a pre-determined area on the depilated back of the rat was cut. The rats were divided into four groups (n=6). Group 1(control) animals were topically applied with simple ointment base, Group 2 animals were topically applied with povidone iodine ointment, Group 3 animals were topically treated with low dose of MCPB (200mg/kg b.w.) mixed with ointment base and Group 4 animals were topically treated with 500 mg/kg b.w. of MCPB mixed with ointment base. Treatments were given once daily till the wound was entirely healed. Contraction rate of wound was monitored by planimetric measurement of the wound area on alternate days, which was achieved by tracing the wound on a graph paper and area is expressed as percentage reduction of the original wound size [15]. Another parameter noted is the Epithelialization period, which is defined as the number of days required for the dead tissue remnants after wounding to fall off leaving no raw wound behind.

Incision wound model

The incision wound study was done according to the method described by Ehrlich and Hunt[16]. The animals were initially anaesthetized under light ether. Incision wound were created on the depilated backs of the animals by cutting two paravertebral incisions of

about 6 cm length through the full thickness of the skin. Interrupted sutures, 1 cm apart, were placed to approximate the cut edges of the skin. The rats were divided into four groups (n=6). Group 1(control) animals were topically applied with simple ointment base, Group 2 animals were topically applied with povidone iodine ointment and, Group 3 and Group 4 animals were topically treated with 200 mg/kg b.w. and 500 mg/kg b.w. of MCPB mixed with ointment base respectively. On 8th day of the post wounding the sutures were removed and on the 10th day, by using continuous water flow technique, skin breaking strength was measured [17].

Dead Space Wound model

Dead space wounds were created through the lumbar region by making a small transverse incision. A polypropylene tube (2.5 × 0.5 cm) was implanted subcutaneously under the dorsal paravertebral lumbar skin[18]. The wound creation day was considered as zero day. The animals were grouped in the same way as it is done in the other two wound model and the respective drugs were administered topically once daily for 10 days. On 10th day the granulation tissue formed on the polypropylene tube was collected by careful dissection and the breaking strength of the granulation tissue was measured. The granulation tissue was oven dried at 60°C overnight and the dry weight was noted. For the determination of the hydroxyproline content, Acid hydrosylate of the dry tissue was used [19].

Biochemical Attributes

The granuloma tissue from the dead-space model was homogenized in phosphate buffered saline (pH 7.0) and centrifuged under cold conditions. The clear supernatant so formed was assayed spectrophotometrically to determine the levels of the antioxidant enzymes, i.e.,superoxide dismutase[20] and catalase[21].

Histopathological studies

The animals were sacrificed after the experiment, and the healed regenerated tissues were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 hrs and processed for paraffin embedding. Sections of 5µ thickness were taken using a microtome. The sections were processed in alcohol-xylene series and stained with haematoxylin and eosin[22] and subjected to histopathological examination

Statistical Analysis

The experimental results were expressed as mean ± S.E.M. Results were analyzed by the one- way ANOVA followed by Tukey-kramer post hoc multiple comparison test using Graph pad InStat version 3.00. P value of <0.05 was considered as statistically significant.

ISSN: 0975-8585

RESULTS AND DISCUSSION

Preliminary phytochemical screening revealed the presence of saponins, flavonoids and tannins. The acute toxicity studies show that the drug was safe up to a maximum dose of 8 g/kg body wt. of the animal.

In excision wound model, the percentage of wound closure was evaluated by recording the changes in wound area at predetermined intervals of time, viz. 3rd, 6th, 9th, 15th and 18th post wounding days. It was observed that wound contracting ability of groups treated with high dose of MCPB was quicker, with a short phase of epithelization, which were similar to the healing potential of povidone treated groups. Groups treated with low dose of MCPB also showed a good wound contraction ($p>0.05$). (Table 1) Histopathological studies of wounds treated with High dose of MCPB showed increase amount of regenerated tissue, epithelization and fibroblast and formation of new blood vessel. The rats with low dose of MCPB promoted epithelization and fibrosis with underlying inflammatory cells predominantly lymphocytes, fibroblasts and new blood vessels. The rats treated with povidone showed regular architecture. The control rats showed ulceration, necrotic debris, neutrophils, lymphocytes and fibroblast.(Fig 1)

Table 1: Effects of MCP extract on excision wounds.

Groups	Treatment	Wound contraction (%)						
		Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Period of Epithelization
Group 1 (control)	PEG ointment base	323.91±1.74 (34.72 %)	295.48±1.62 (41.80 %)	241.12±1.14 (51.12 %)	172.32±2.45 (65.35 %)	102±2.56 (79.62%)	52±3.21 (89.92%)	24.51±2.56
Group 2 (Standard)	Povidone Iodine ointment	285.02±2.21 (43.20%)*	237.23±3.54 (52.3 %)*	162.24±2.95 (67.62 %)*	97.45±3.24 (80.65 %)*	49.23±3.52 (90.06 %)*	22±2.92 (95.64 %)	20.42±1.79
Group 3 (Low Dose)	200mg/kg of MCPB extract	290.05±1.20 (42.32 %)*	254.32±3.24 (49.23 %)	186.24±2.84 (62.81 %)*	112.21±2.21 (77.61 %)*	85.21±2.32 (83.62 %)*	35±3.26 (93.23%)	21.32±2.12
Group 4 (High Dose)	400mg/kg of MCPB extract	251.12±2.12 (49.81%)*	203.02±3.21 (59.42%)*	147.23±2.13 (70.63%)*	86.25±2.84 (82.85%)*	39.24±3.54 (92.23 %)*	12±1.63 (97.63%)*	19.21±0.95

All the results are reported as mean ± SEM. One way ANOVA and* $p<0.01$ is considered significant. Figures in Parenthesis indicate % wound contraction

The incision wound study was carried out to assess the tensile strength of the regenerated tissue. The High dose of MCPB treated group showed a major breaking strength which was almost similar to the povidone iodine treated group. Low dose of MCPB treated group also showed better breaking strength when compared to the normal.(Table 2) Histopathological sections of incision wounds exposed to High dose of MCPB showed granulation tissue with plenty of fibroblasts and thick bundles of collagen tissue, which was similar with the standard drug povidone iodine. The rats treated with low dose of MCPB showed granulation tissue macrophages and fibroblasts. Povidone treated group of rats showed normal histopathological architecture. The control group of rats showed a mild re-epithelialization with chronic inflammatory cells. (Fig 2)

ISSN: 0975-8585

In dead space wound model significant increase in glanuloma weight is observed in rats treated with MCPB and a significant breaking strength were observed. Studies on the estimation of antioxidant enzymes disclose that the extract MCPB significantly augment the levels of superoxide dismutase and catalase, the two powerful antioxidant enzymes of the body that are recognized to quench superoxide radicals. (Table 2).

Table 2: Effects of MCPB on incision wound and Dead space model

Groups	Treatment	Incision wound model	Dead space model				
		Tensile strength (g)	Granuloma weight (g/100g)	Breaking strength (g)	Hydroxyproline content (mg/100g)	Superoxide dismutase (IU/mg)	Catalase (IU/mg)
Group 1 (control)	PEG ointment base	229.36 ±1.21	11.24 ±0.36	235.42 ±2.13	590.82 ±7.50	0.117 ±0.011	2.44 x 10-2 ± 2.3 x 10-3
Group 2 (Standard)	Povidone Iodine ointment	393.25 ±2.41***	31.24 ±0.36***	398 ±1.42***	719.94 ±0.85***	0.222 ±0.016***	6.82 x 10-2 ± 5.7 x 10-3***
Group 3 (Low Dose)	200mg/kg of MCPB extract	372.23 ±0.74***	25.24 ±0.45***	368 ±1.43***	679.64 ±0.32***	0.182 ±0.021**	6.34 x 10-2 ± 4.3 x 10-3***
Group 4 (High Dose)	400mg/kg of MCPB extract	400.32 ±0.25***	31.34 ±0.25***	402 ±2.12***	725.67 ±0.36***	0.232 ±0.023***	7.12 x 10-2 ± 3.2 x 10-3***

N=6, Values are Mean ± SEM, *** p<0.001 Vs control, ** p<0.01 Vs control

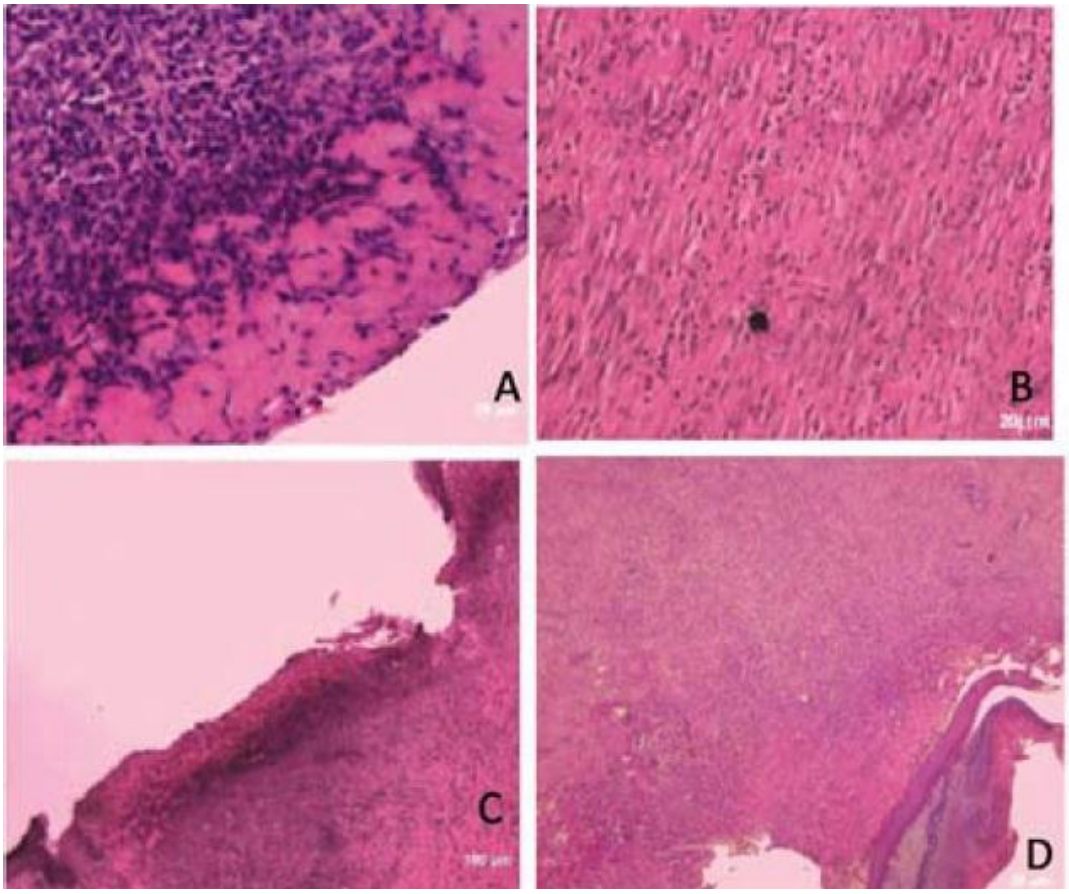

Fig 1: Microscopic observation of Excision model; A. Control Group, B. Standard group treated with Povidone iodine, C. Group treated with low dose of MCPB(200mg/kg), D. Group treated with high dose of MCPB (500mg/kg)

ISSN: 0975-8585

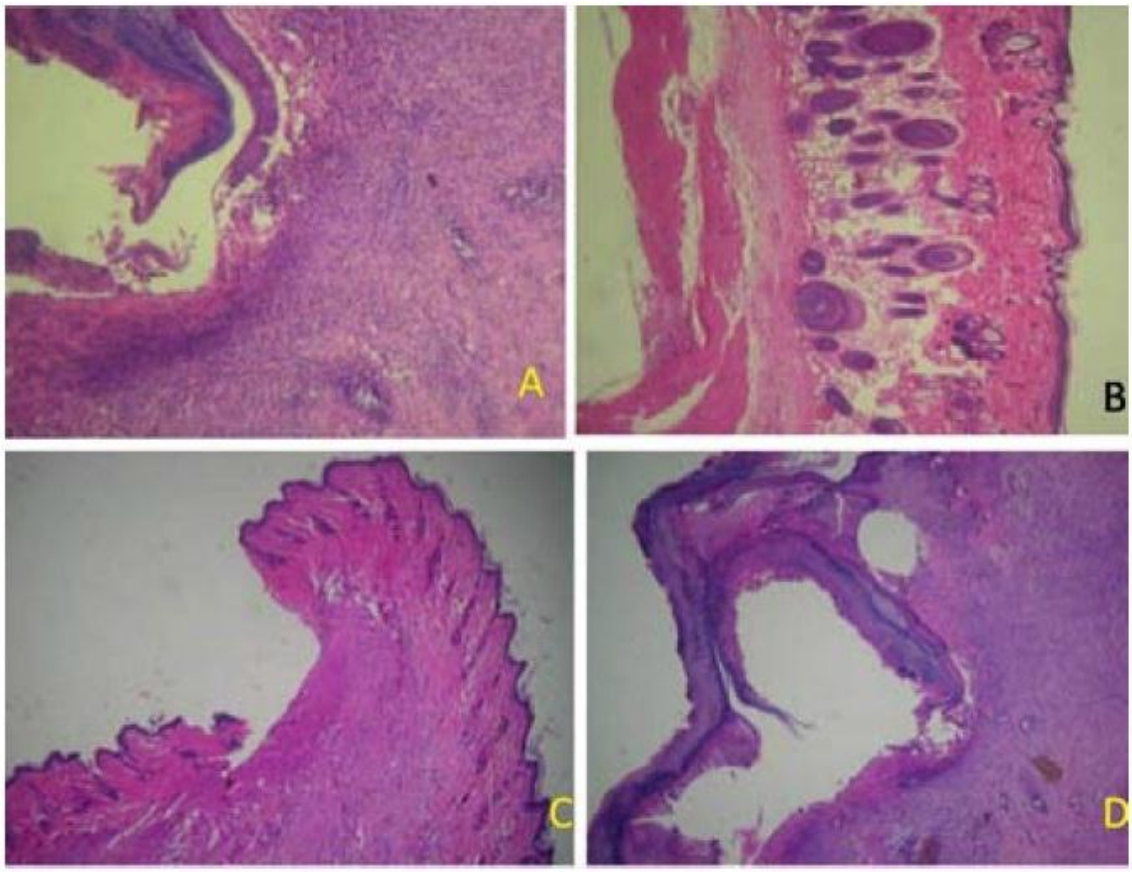

Fig 2: Microscopic observation of Incision wounds, A. Control Group, B. Standard group treated with Povidone iodine, C. Group treated with low dose of MCPB (200mg/kg), D. Group treated with high dose of MCPB (500mg/kg)

DISCUSSION

Wound healing is a complex phenomenon and it mainly comprises of 3 phases: inflammatory Phase, Proliferative phase and Maturational phase. In inflammatory phase the wound is characterized by hemostasis and inflammation, which is followed by the proliferation phase that comprises of the process like epithelization, angiogenesis, and collagen deposition. The final phase is the Maturational phase, where the wound undergoes contraction ensuing in a smaller amount of noticeable scar tissue.

Granulation tissue formed in the final part of the proliferative phase after the topical application of MCPB at both low dose (200mg/kg) and High dose (500mg/kg) is composed of fibroblasts, collagen and new blood vessels. The increase in dry granulation weight in the test animal groups indicates higher protein content. Although the high dose (500mg/kg) MCPB treated animals shows better results than the reference drug Povidone treated group, both the dose of MCPB treated group showed a significant increase in hydroxyproline content. Collagen is composed of hydroxyproline and amino acids. Hydroxyproline has been used as a biomarker for tissue collagen and the collagen is the major components which supports and strengthen the extracellular tissue.

In studies using the excision wound model, the animals were treated with MCPB, which showed a significant decrease in the epithelization period, as evidenced by shorter

period for the complete closure of wound as compared to the control. The drug also facilitates the rate of wound contraction significantly at both the dose level.

Estimation of antioxidant showed that MCPB increased the levels of superoxide dismutase and catalase, which are the two powerful component of the body that are known to quench superoxide radicals. Comparing the results of phytochemical screening with the estimation of antioxidents, it suggests that tannin may be one of the active components responsible for the antioxidant property. Thus the enhanced wound healing may be due to the free radical scavenging action of the plant.

CONCLUSION

The present study clearly showed that the MCPB has properties that render it capable of promoting accelerated wound healing property as compared to the control. As compared to the standard (Povidone iodine), the high dose of MCPB (500mg/kg b.w) showed a predominantly better wound healing activity. Low dose of MCPB (200mg/kg b.w) although did not showed better result than the standard but its property of wound healing is comparable to the standard drug (povidone iodine).

ACKNOWLEDGEMENT

The authors are grateful to 'The oxford college of pharmacy', for providing all the animals and the necessary facilities for the completion of our work. We would also like to acknowledge our sincere thanks to Dr. Padma M Parak, Principal, The oxford college of pharmacy for her guidance.

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