Journal of Ethnopharmacology 121 (2009) 338–341

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jethpharm

Ethnopharmacological communications

Wound healing properties of *Carica papaya* latex: *In vivo* evaluation in mice burn model

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ARTICLE INFO

Article history:
Received 7 August 2008
Received in revised form 21 October 2008
Accepted 30 October 2008
Available online 8 November 2008

Keywords:
Carica papaya
Latex
Burn wound
Hydrogels

ABSTRACT

Ethnopharmacological relevance: Carica papaya is traditionally used to treat various skin disorders, including wounds. It is widely used in developing countries as an effective and readily available treatment of various wounds, particularly burns.
The aim of the study: This study was aimed at investigating the healing efficiency of papaya latex formulated as 1.0 and 2.5% hydrogels.
Materials and methods: Burns were induced in Swiss albino mice divided into five groups as following; Group-I (negative control) received no treatment. Group-II was treated with Carbopol 974P NF empty gel. Groups-III and -IV were treated with Carbopol gel containing 1.0 and 2.5% of dried papaya latex, respectively. Group-V (positive control) received the standard drug (silver sulphadiazine and chlorhexidine gluconate cream). The efficacy of treatment was evaluated based on the hydroxyproline content, wound contraction and epithelialization time.
Results: Hydroxyproline content was found to be significantly increased in the Group-III. Significant increase in percentage wound contraction was observed from day 12 in Group-IV and from day 20 in Groups-III and -V. The epithelialization time was found to be the shortest in Group-IV.
Conclusion: It may be concluded that papaya latex formulated in the Carbopol gel is effective in the treatment of burns and thus supports its traditional use.

1. Introduction

Burns and trauma wounds are very common in both developed and developing countries, however, in developing countries burns constitute a major health problem because the incidence of severe complications is high and financial resources are limited. About 42.0% of the total population of Nepal is lying below the poverty line. Moreover, due to a high dependency on limited resources like electricity, gas and water, most of the people have to rely on firewood for cooking and other household activities and thus are exposed to the open fire which again increases the incidence of burning (Anonymous, 2003). The use of traditional medicinal remedies and plants in the treatment of burns and wounds is an important aspect of health treatment and at the same time a way to reduce the financial burden. Several plants used as traditional healing remedies have been reported to treat skin disorders, including burn wounds (Starley et al., 1999; Biswas and Mukherjee, 2003; Inngjerdingen et al., 2004; Mikhalchik et al., 2004; Muthu et al., 2006; Nayak and Pinto Pereira, 2006; Singh et al., 2006).

Our particular interest was *Carica papaya*, traditionally used to treat skin disorders (Mikhalchik et al., 2004). For example in Gambia, papaya fruit has been used in full thickness and infected burns and has shown promising results in clinical trials (Starley et al., 1999). *Carica papaya* (family Caricaceae) is cultivated in tropical and subtropical regions all around the world as fruit due to its palatable taste, nutritive value and easy digestion (Monti et al., 2004). Damaging the papaya tree inevitably severs its laticifers, eliciting an abrupt release of latex (Azarkan et al., 2003). The latex from unripe papaya fruits contains a mixture of cysteine endopeptidases such as papain, chymopapain A and B, papaya endopeptidase II, papaya endopeptidase IV, omega endopeptidase (Azarkan et al., 2003), chinitases, protease inhibitors, linamarase and proteins without known functions (Azarkan et al., 2004; Oloyede, 2005). Although papaya fruit is known to posses wound healing properties, no systematic studies have been carried out up to now on the clinical evaluation of the wound healing potential of *Carica papaya* latex. Thereof, its effects were investigated using thermal wound models in mice. Papaya latex was applied to the burn wounds using hydrogel as a vehicle system, known to have superior properties

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doi:10.1016/j.jep.2008.10.030

such as spreadability, stability and cooling effects (Sankalia et al., 2005).

2. Materials and methods

2.1. Plant material

Papaya latex (*Carica papaya*, Caricaceae) was identified by comparing the authentic sample and confirmed with the help of expert (Professor Dr. Kayashta, Tribhuvan University, Nepal). The voucher specimen is (No. 283) preserved in the Museum of Natural Medicine of The School of Pharmaceutical and Biomedical Sciences, Pokhara University, Nepal. The latex was collected locally in the early mornings (7.00–8.00 am), as the flow of latex is low during the day. Collection was done by making 1–2 mm deep vertical incisions on the skin of unripe but mature fruit. The latex was then dried at room temperature till it became crumbly and non-sticky. The dried latex was triturated using a mortar and pestle and sieved through a mesh size 170. It was stored at 4–8 °C until use.

2.2. Preparation of gels

The gel was prepared following the methods described by Škalko et al. (1998) and Pavelic et al. (2001). Carbopol 974P NF (0.25 g: Goodrich, USA) was dispersed in 22 g of distilled water and mixed by stirring continuously in a magnetic stirrer (Yamato Scientific Co. Ltd., Japan) at 800 rpm for 1 h. Glycerol (1.25 g) was added to the mixture under continuous stirring. The mixture was neutralized by drop-wise addition of 50% triethanolamine (w/w). Mixing was continued until a transparent gel was formed. Three types of gel formulations were prepared viz. empty gel, gel containing 1.0% (w/w) latex and gel containing 2.5% (w/w) latex. Silver sulphadiazine and chlorhexidine gluconate cream (1.0% w/w) manufactured by Apex Pharmaceuticals (P) Ltd., Birgunj, Nepal was used as a standard treatment for positive control.

2.3. In vivo healing evaluation

2.3.1. Animals

Male Swiss albino mice (total forty) weighing 35–45 g were procured from the Department of Plant Research, Kathmandu. The animals were approximately 4 weeks old. They were individually housed in clean polyethylene cages under standard experimental conditions of temperature 23 ± 2 °C, 12 h light/dark cycle and fed on normal pellet diet and water *ad libitum*. The mice were used for the experiment after one week of acclimatization period. The experiment was conducted according to the Ethical Guidelines for Care and Use of Animals in Health Research in Nepal.

2.3.2. Wound evaluation

The animals were anesthetized with diethyl ether and their dorsal surface was shaved with a sterile blade. The shaved area was disinfected with 70% (v/v) ethanol. The burn wounds were created using the method described by Rozaini et al. (2004) with some modifications. A cylindrical metal rod (10 mm diameter) was heated over the open flame for 30 s and pressed to the shaved and disinfected surface for 20 s in mice under light diethyl ether anesthesia. The animals were randomly divided into 5 groups each containing 4 animals. Group-I (negative control) received no treatment at all. Group-II was treated with empty Carbopol 974P NF gel. Groups-III and -IV were treated with gels containing 1.0 and 2.5% dried papaya latex, respectively. Group-V (positive control) received the standard drug (silver sulphadiazine and chlorhexidine gluconate cream; 1.0% w/w). The treatments (100 mg/mouse) were applied topically once a day, starting from the wound induction until complete healing. The parameters studied were the percentage wound contraction and the epithelialization time. In a separate experiment, hydroxyproline content was determined. Twenty mice, divided in five groups, received the treatments in a same manner as described for determination of the percentage wound contraction and epithelialization.

2.3.3. Measurement of wound area

The progressive changes in wound area were measured in mm^2 by tracing the wound boundaries on a transparent paper on every 4-day interval. The wound areas in all groups were recorded on a graph paper. Wound contraction was expressed as reduction in percentage of the original wound size.

2.3.4. Determination of the hydroxyproline content

On the 11th day, the animals from each group were euthanized using diethyl ether and used to determine hydroxyproline content. The protein content of the tissue was estimated using the techniques described by Neuman and Logan (1950). The wound tissue was excised and its weight recorded. The tissue was dried in oven at 60 °C for 12 h and the dry weight was again noted. They were hydrolyzed in 6 N HCl for 24 h at 110 °C in sealed glass tubes. The hydrolysate was neutralized to pH 7.0. The samples (200 μl) were mixed with 1 ml of 0.01 M $CuSO_4$ followed by the addition of 1 ml of 2.5 N NaOH and then 1 ml of 6% H_2O_2. The solution was mixed and shaken occasionally for 5 min. All the tubes were incubated at 80 °C for 5 min with frequent vigorous shaking. Upon cooling, 4 ml of 3 N H_2SO_4 was added with agitation. Finally, 2 ml of 5% p-dimethylaminobenzaldehyde was added. The samples were incubated at 70 °C for 16 min, cooled by placing the tubes in water at 20 °C, and the absorbance was measured at 500 nm using a digital photo colorimeter (EI Products, India). The amount of hydroxyproline in the samples was calculated using a standard curve prepared with pure L-hydroxyproline at the same time.

2.4. Statistical analysis

Experimental data are expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using Student's paired *t*-test as the difference between pair of groups. Data were considered significant at $p < 0.05$.

3. Results

3.1. Hydroxyproline content

The measurement of hydroxyproline can be used as an index for collagen turnover (Nayak and Pinto Pereira, 2006). Increase in hydroxyproline content indicates increased collagen synthesis which in turn leads to enhanced wound healing. In our study, the hydroxyproline content was found to be significantly increased in Group-III ($p < 0.05$) as compared with the Group-I (Fig. 1). The content was found to be increased in the Group-IV as well, but due to larger variation among animals, the difference was not significant.

3.2. Wound contraction

Wound contraction is another parameter used to assess wound healing. Significant wound contraction was initiated from day 12 in the Group-IV ($p < 0.05$). Highly significant wound contraction was observed from day 20 in Groups-IV and -V ($p < 0.01$) and Group-III ($p < 0.05$; Fig. 2).