An Investigation on Burn Wound Healing in Rats with Chitosan gel Formulation containing Plant Enzymes

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Abstract- Plants and their extract have immense potential for the management and treatment of burn wounds. The plant enzyme induces wound healing and regeneration of lost tissue by multiple mechanisms. Various studies have shown that chitosan is effective in promoting wound healing. In this study, we aimed to develop an effective topical gel formulation containing plant enzymes (papain and bromelain) and chitosan as a gelling agent. In in-vivo studies, animals were divided into six groups as follows: Group- I (negative control) received no treatment, Group-II was treated with empty chitosan gel, Group III, IV and V were treated with Chitosan gel containing papain, bromelain and papain – bromelain mixture respectively, VI- (positive control) received the standard drug (Framycetin Sulphate IP). Healing was assessed by the rate of wound contraction and period of epithelization. Our data suggest that chitosan gel containing plant enzymes shows promising effects in burn wound healing.

Keywords – Plant enzymes, Chitosan, Burn, Wound healing

I. 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 [1].They are causing morbidity and mortality of millions of people worldwide [2].The use of traditional medicine 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 and plant products have been reported to treat skin disorders including burn wounds [3-6]. The process of wound healing occurs in different phases such as coagulation, epithelization, granulation, collagenation and tissue remodeling [7, 8].

Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4) linked D-glucosamine and N-acetyl-D-glucosamine units. Commercially it is produced by the exhaustive deacetylation of chitin, a structural element in the exoskeleton of crustaceans and insects, which is the second most abundant natural biopolymer after cellulose [9]. Based on ionic interactions between the cationic primary amino groups of chitosan and these anionic substructures of the mucus, mucoadhesion can be achieved which is essential for topical use [10].A role of chitosan in the wound healing process has been demonstrated through haemostatic action, macrophage activation, stimulation of cell proliferation and histo-architectural tissue organization [11]. Chitosan gel also acts as an ideal wound dressing. It is biocompatible, biodegradable, hemostatic, anti-infective and accelerates wound healing [12].

The major characteristic of burns is the formation of an eschar, which is made up of burned and traumatized tissue. The eschar also serves as a medium for bacterial growth, hence a source of infection, contamination and sepsis. As a result prompt removal of eschar is essential for healing of burns [13]. Plant enzymes used in this study shows promising effects for eschar removal.

Papain, a major component of latex from *Carica papaya* is a nonspecific cysteine proteinase that is capable of breaking down a wide variety of necrotic tissue substrate over a wide pH range from 3.0 to 12.0 this may also contributed for faster burn wound healing and facilitated by the action of proteinases [14]. Furthermore, it can be used to increase chitosan wound healing capability and also shows anti-inflammatory, anti-bacterial and antioxidant property [15]. It is also useful in reducing bacterial burden, decreasing exudates and increasing granulation tissue formation [16].

Bromelain is a mixture of proteolytic enzymes that is derived from the stem of the pineapple plant *Ananas cosmosus* proteolytically removes certain cell surface molecules that affect lymphocyte migration and activation in burn wound healing [17]. Bromelain can effectively decreases neutrophil migration to site of acute inflammation and support the specific removal of the CD128 chemokine receptor for anti-inflammatory activity [18]. It also shows antioxidant activity which is essential for wound healing [19].

II. MATERIALS AND METHOD

Materials

Papain kindly provided as a gift sample by Advanced Enzymes Technology Ltd. Thane (W), India, Bromelain purchased from Prisha Herbals, Indore, India and Chitosan obtained from Sigma Aldrich, Mumbai, India. All other chemicals and solvents were of analytical grade.

Preparation of the chitosan gel

Gels were prepared by adding glacial acetic acid (1% v/v) into half of required water. The weighed amount of chitosan was added in above solution and stirred slowly. After the swelling remaining amount of water was added and mixed. Methyl paraben sodium salt (0.1% w/w) was added as preservative. After the preparation of gel required amount of plant enzymes were added. The resulting gels were stirred and sonicated to remove air bubbles.

Design of animal experiment

All animal experiments were conducted under the protocol approved by Institutional Animal Ethical Committee (IAEC) (Proposal No. KNCOP/R&D/AN-PROT/14-15/05) and according to guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India. Male albino wistar rats weighing 150-200gm were used for the present study. Rats were housed in clean polyethylene cages separately under standard environmental conditions, fed on normal diet and water *ad libitum*. The animals were divided in six groups as shown in Table 1.

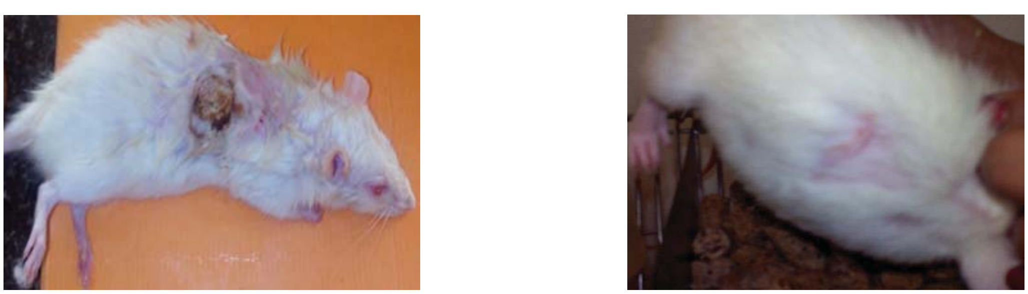
Table 1: Grouping of animals for experimental design

Group	Treatment
Group- I	Negative control received no treatment
Group- II	Treated with empty chitosan gel
Group- III	Treated with chitosan gel containing papain
Group- IV	Treated with chitosan gel containing bromelain
Group- V	Treated with chitosan gel containing papain-bromelain mixture
Group- VI	Positive control received standard drug (Framycetin Sulphate IP).

Formation of the burn wounds

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 by using a cylindrical metal rod (10 mm diameter) previously heated over the open flame for 30s and pressed to the shaved and disinfected surface for 20s in rat under light diethyl ether anesthesia [20].Formulations were applied topically once a day, starting from wound induction until complete healing. The parameters studied were the percentage wound contraction, epithelization time and hydroxyproline content (Fig.1)

Figure 1 Photographs of macroscopic appearance of burn wound on 0 and last day



Measurement of wound area

A wound contraction, which contributes for reduction in percentage of the original wound size, was studied by tracing the raw wound. 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; similarly number of days for complete epithelization was noted [21].

Determination of hydroxyproline content

On the 11thday, the animals from each group were euthanized using diethyl ether and used to determine hydroxyproline content. The wound tissue was excised and its weight recorded. The tissue was dried in oven at 60^{o}Cfor 12 hr and the dry weight was noted. They were hydrolyzed in 6 N HCl for 24 h at 110^{o}C in sealed glass tubes. The hydrolysate was neutralized to pH 7.0. The samples were mixed with 1 ml of 0.01M $CuSO_4$ followed by the addition of 1 ml of 2.5N 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 ^{o}C for 5 min with 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 ^{o}C for 16 min, cooled by placing the tubes in water at 20 ^{o}C, and the absorbance was measured at 500 nm [1].

Statistical analysis

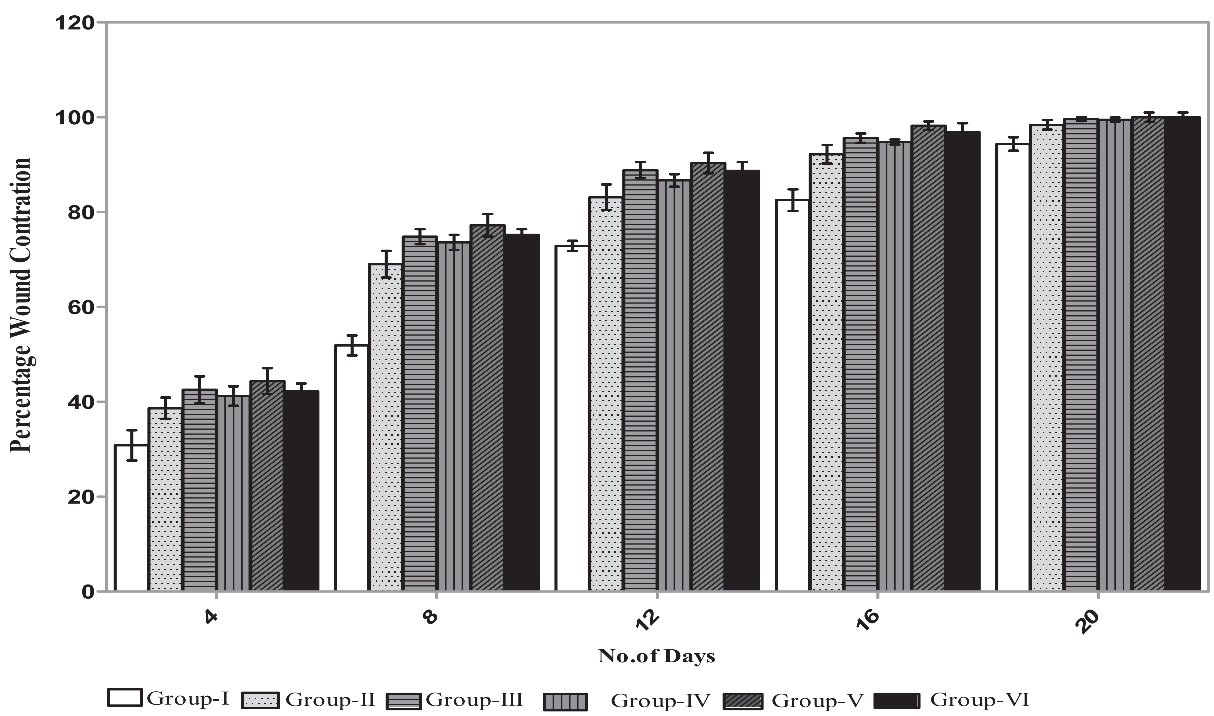
Experimental data was expressed as mean ± S.D. Statistical analysis of data was performed using one way-ANOVA. Probability (p) value is estimated by using Instat Graph Pad prism, window (USA)

III. RESULT

Rate of wound contraction

The rate of wound contraction is major parameter for evaluation of wound healing activity. The significant wound contraction was found in group treated with chitosan gel containing plant enzymes as compared to untreated group; however gel containing mixture of plant enzymes shows highest wound contraction among all other groups. (Fig.2)

Figure 2.Effect of topical application of gel on burn wound expressed as percentage wound contraction. N=6, Values are Mean ± S.D., ($P < 0.05$ vs. Group-I)



Epithelialization time

The epithelialization time is time required for complete epithelialization of wounded tissue. It was found to be significantly reduced in group treated with chitosan gel containing plant enzymes as compared to untreated group; however group treated with gel containing mixture of plant enzymes shows reduced epithelialization time among all other groups. (Table-2)

Table 2: Effect of topical gel on epithelialization time

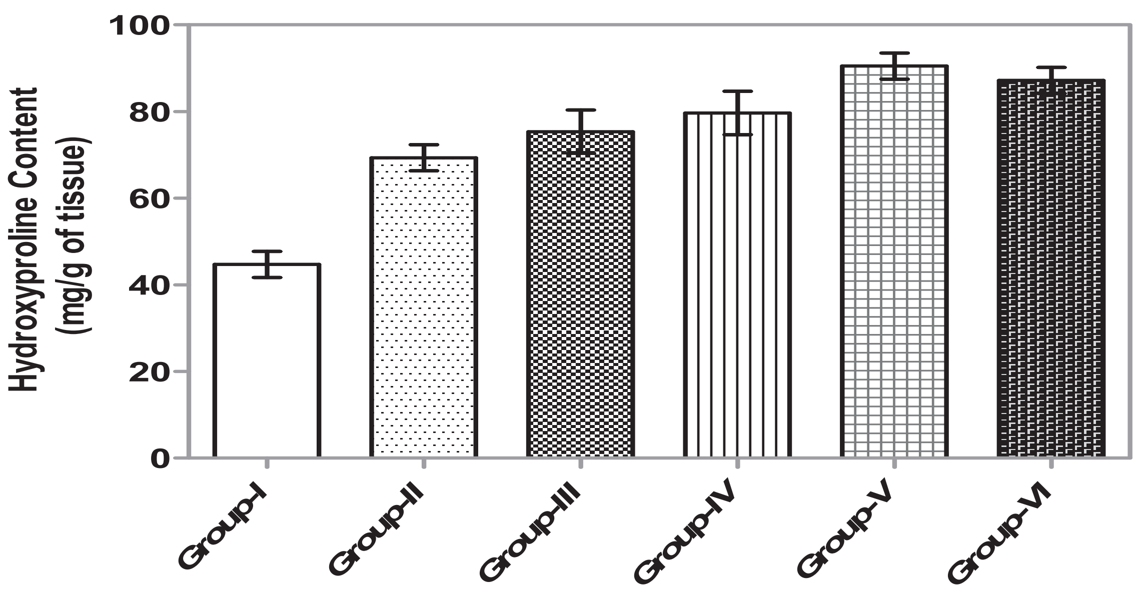
Group	Epithelialization time
Group- I	25.166 ± 1.72
Group- II	21.666 ± 1.03
Group- III	20.05 ± 1.04
Group- IV	19.666 ± 1.03
Group- V	18.666 ± 1.21
Group- VI	19.166 ± 0.75

N=6. Values are Mean ± S.D. ($P < 0.01$ vs. Group-I)

Hydroxyproline content

Measurement of hydroxyproline content can be used as an index for collagen synthesis. More hydroxyproline released by tissue indicates more collagen synthesis which leads to enhanced wound healing. In present study, hydroxyproline content in treated group was found to be significantly increased as compare to untreated group: however group treated with gel containing mixture of plant enzymes shows increased hydroxyproline content among all other groups (Fig.3)

Figure3. Effect of topical application of gel on Hydroxyproline content, N=6, Values are Mean ± S.D., ($P < 0.001$ vs. Group-I)



III. CONCLUSION

Burn wounds have a complex healing process, cause severe discomfort and are prone to infection and other complications. The study of the physicochemical interactions of chitosan with the most important components of living matter appears to be an essential point for the interpretation of biological responses induced by the presence of chitosan in living system. Although several studies have investigated the accelerating effects of wound healing by chitosan in multiple animal models, most of the results were considered inconclusive. The resultant wound healing effects were, in many cases, attributed to the synergistic effect of the numerous materials.

The scope of present study, however, was to determine the effects of chitosan gel with plant enzymes on the extent of burn wound healing acceleration. Papain and bromelain are known to be effective in disloughing necrotic tissue, prevention of infection and the antimicrobial and antioxidant properties related to hydroxyl scavenging. Moreover, they decrease the risk of oxidative damage to tissue. Wound contraction, the process of shrinkage of area of wound, depends on the reparative abilities of tissue, type and extent of the damage. In present study, the wound contraction was significantly faster and higher in animals treated with gels containing plant enzymes (Fig 2).

Collagen, the major protein of the extracellular matrix, is the component that ultimately liberates free hydroxyproline. Therefore, measurement of hydroxyproline can be used as an index for collagen synthesis. In present study, hydroxyproline content in treated group was found to be significantly increased as compare to untreated group: however group treated with gel containing mixture of plant enzymes shows increased hydroxyproline content among all other groups (Fig.3). Finally, the epithelialization time was also found to be shorter in animals treated with chitosan gel containing plant enzymes as compared to untreated group (Table-2).

Moreover, the role of chitosan gel as a suitable vehicle for delivery of plant enzymes cannot be neglected owing to its adhesive properties. It may be therefore concluded that plant enzymes (papain and bromelain) shows synergistic burn wound healing effect with chitosan as depicted by faster wound contraction, shortening of epithelialization time and increased hydroxyproline content. Hence the result supports the traditional use of plant enzymes in skin disorders including burn wound healing.

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