The Effect of Cast Films of Bovine Mucin and Prosopis Gum Containing a Cicatrin® Powder on Wound Healing

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ABSTRACT: The aim of this work is test the effect of cast film of bovine mucin and prosopis gum containing cicatrin powder on wound healing in rats. Mucin was processed from the small intestine of freshly slaughtered cow via precipitation with chilled acetone, air-drying and pulverization. Prosopis was extracted using acetone; the film was formulated by a mixture of bovine mucin and prosopis gum. The formulations were used in the treatment of wound inflicted on the animal and this was compared to the standard antibiotics cicatrin used in wound healing. In all cases, there was a progressive decrease in wound area indicating an efficacy of the samples in healing. By the 15th day, the sample containing 0.2 g of the mixture of bovine mucin, prosopis and cicatrin powder showed 100 % healing. Th negative control show 65 %, prosopis alone showed 50 %, bovine mucine alone showed 90 % and cicatrin alone showed 70 %. Increase in the mucin concentration gave a remarkable improvement. These results suggest that a mixtures of bovine mucin, cicatrin and prosopis gum has a better healing effect than cicatrin powder alone.

Key words: mucin, Prosopis gum, cicatrin, healing

INTRODUCTION

Wound healing is an important biological process involving tissue repair and regeneration. A wound is described as a break in the continuity of tissue, from violence or trauma and is regarded as healed if there is restoration to the wound or inflamed tissue to normal condition. Wound healing can be by primary, secondary and third intention depending on the nature of the edges of the healed wounds.[1] There are four distinct stages of wound healing namely - inflammatory stage, debridement stage, proliferation stage and maturation/remodeling stage.[2] Some factor play major roles in wound healing such factors are: bacteria infection, nutritional deficiency, drugs, site of wound, and heath condition of patient.[3] Several agents has been used in wound healings these range from antibiotics especially in post operative surgery, the simple reason is to prevent secondary infection and to aid early healing. Other includes the uses of plants and plant material such as prosopis gum has since been explored in folklore. Also the use of bio-material such as mucin and honey has also been investigated.

Mucin or mucus glycoproteins are a family of polydisperse molecules, which carry out multiple tasks at mucosal surface throughout the body. They contribute to the mucus gel barrier and are part of the dynamic, interactive mucosal defensive system.[4] Mucus is a very high molecular weight, carbohydrate rich protein with up to 80 % O-glycosidically linked carbohydrate. Several studies carried out on mucus glycoproteins from many organs have suggested that these macromolecules consist of subunits held together by interchain disulphide bonds and further stabilized by non-covalent interaction. The end result

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of multiple interconnections is an extended and random network, which imparts to mucus secretions their characteristic property of viscoelasticity. Evidence that S-S bond play an important structural role has been provided by demonstrations that thiol-group reagents decrease the viscosity and increase the solubility of the native mucus secretions[5] and in some cases decrease the molecular weight of purified mucin. For example Starkey et al.[5] observed that the sedimentation coefficient of a large porcine gastric mucin decreased from 335 MW to 145 MW after treatment with 0.2 M of 2-mercaptoethanol. Somewhat similar but less dramatic effects have been observed after reduction of bronchial mucus glycoproteins.[6]

The use of prosopis Africana belonging to the family fabaceae is being used traditionally as medicine in many African homes. These includes: the leaves are used in treatment of headache and toothache, leaves and bark are combined in the treatment of rheumatism, skin disease and eyewashes, the roots are used as diuretic, and in the treatment of dysentery, bronchitis and stomach cramps.[7] The prosopis gum has been used in the present day research as bio-adhesive agent in delivery of metformin, this show a synergistic effect.[8] Prosopis gum can be used to treat infection skin irritation and in the management of wound.[9]

Hence this study is to evaluate the effect of mixture of bovine mucin, prosopis gum and cicatrin powder in wound healing.

MATERIALS AND METHOD

Materials

The following materials were purchased from their local suppliers and used without further purification. Cicatrin powder (Glaxo, Nigeria), citric acid, sodium hydroxide (Merck), acetone (BDH, England), concentrated HCl, paraldehyde injection (Kamala, India), diazapam (Roche, England), sodium chloride (Merck, Germany), distilled water prepared from an all-steel still (Kottermann, England). All other reagents were of analytical grade and were used as such.

Animals. Mature wistar albino rats of both sex of an average weight of 130 g obtained from the Department of Biochemistry, University of Nigeria and fed on 'chicks marsh'(Top Feed, Nigeria) were used for the study. After the purchase, all the rats were allowed to equilibrate in standard conditioned animal houses at the Department of Biochemistry, University of Nigeria for a period of one week before use.

Methods

Extraction of bovine mucin. The small intestine of freshly slaughtered cow was obtained from the Nsukka abattoir and dissected starting from the beginning of the jejunum to the ileocaecal sphincter. The intestines, sectioned into short lengths, were flushed through with chilled saline, and the mucosal surface was exposed by longitudinal dissection. By using a microscope slide, the mucus layer was gently scraped off. The mucus was precipitated using chilled acetone. The resultant flakes were pulverized and stored in an air-tight container until used.

Preparation of Prosopis Gum. A water-soluble gum was extracted from the endocarp capsule of the seed coat of Prosopis africana. The gum preparation varied substantially with the method used. In this study dehulling method was used.

The seeds were soaked in water for 24 h and cooked for 4 h using glass containers to prevent the darkening that takes place in metallic containers. The swollen tegument were collected manually and soaked in an aqueous solution of 0.1 % w/v acetone for 24 h. At the end of this period, the material was homogenized. The lightly viscous material obtained was passed through a muslin cloth to remove any gritty particles and the filtrate was precipitated with twice the volume of acetone. The material was dried in a desiccators containing Agar gel (to prevent auto-oxidation) for 24 h and pulverized using an end-runner mill. The powder sample was stored in tightly closed containers until used.

Film preparation. The film were cast from a solution of different concentrations of bovine mucin, prosopis gum and cicatrin powder as shown below, using solvent evaporation technique.

Prosopis gum	0.2 g
Bovine mucin + Prosopis gum	0.2 g each
Bovine mucin + Prosopis gum	0.4 g each
Bovine mucin + Prosopis gum + Cicatrin powder	0.2 g each

Films of prosopis gum alone were prepared with hot water below 100 ^{0}C which serve as solvent. Different concentrations of prosopis gum were put in the cylinder turned clockwise directions with stirring on gradual addition of hot water till it dissolved in the solvent (hot water). Films were carefully cast on a 20 cm diameter Petri dish, after allowing the solvent to evaporate for 24 h. The films were removed from the plates and subsequently air dried for an additional 24 h. The prepared films were carefully cut into strips, before application on the wound inflicted on rats.

The same process was applied in the film preparation of bovine mucin + prosopis gum and in the film preparation of Bovine mucin + prosopis gum + cicatrin® powder.

Preparation of the wound sites in the experimental animal. The sites of the wound were prepared by the excision wound model.[10] The animals were anaesthetized with 8 mg/kg diazepam, the hairs on the skin back were shaved with scissors and the area of the wound created was outlined on the back of the animals with a permanent marker. A circle of the diameter 14 mm was marked on each of the two sides on the skin using toothed forceps, a surgical blade and pointed scissors. Then circular incisions were made on the marked area of the skin surface and the skin carefully dissected out. The area was measured immediately to ensure accuracy by tracing out the wound area using a transparent tracing paper and the area was recorded.

Determination of wound healing effect of the films. Treatment with the film of various concentrations of bovine mucin, prosopis gum and cicatrin was started shortly after wound was made on the animal once daily. Group 1 serve as negative control and treated with normal saline alone, group 2 served as reference standard and considered as the positive control and treated with 0.2 g of cicatrin® powder at different concentration with 100 mg of vaseline jelly was used as bioadhesive agent, because of inability of cicatrin powder to form film. Group 3 were treated with different concentration of bovine mucin (0.2 g) alone with 100 mg vaseline jelly was used as bioadhesive agent in bovine mucin because bovine alone cannot make a film; group 4 was treated with films of different concentration of prosopis gum alone (0.2 g); group 5 was treated with the films of different concentration of bovine mucin (0.2 g) plus the different concentrations of prosopis gum (0.2 g); group 6 was treated with films of the different concentration of bovine mucin (0.4 g) plus films of different concentration of prosopis gum (0.4 g). Then the group 7 animals were treated with films of bovine mucin (0.2 g) plus prosopis gum (0.2 g) plus cicatrin® powder (0.2 g).

All medications were applied once daily, after dressing the wound with methylated spirit. The parameters studied were the wound closure, the measurements of the wound areas were taken on 3rd, 6th, 12th, 15th using transparent paper and a permanent marker.

Data analysis. The data were subjected to the student's t-test at the 5 % level of significance.

RESULTS AND DISCUSSION

The results of the wound healing effect of cast films of bovine mucin and prosopis gum containing cicatrin® powder are shown in Table 1. There was a general decrease in wound area with time and by the 15th day the wound area of animals in group VII, came to zero while the rest groups except prosopis has above 70 % healing rate, which shows that it have rapid healing rate.

Animal treated with mucin alone in group III, shows a remarkable healing effect than group treated with prosopis or cicatrin alone as seen in group II and IV. Cicatrin® which is the standard drug use has

greater anti-bacterial property to compare with prosopis gum and lesser when compared with mucin.

Combination of mucin and prosopis as seen in group V, has no advantage compare to when mucin alone was used. Researcher has shown that the healing effect of mucin in group III, could be attributed to it high antibacterial activity that is said

Table 1. Percentage wound healing of the test samples

Group	Percentage wound healing (mean ± sem) after days of post surgery (mm)					
	0	3rd	6th	9th	12th	15th
I (N)	0.00	17.00 ± 0.35	23.20 ± 0.35	51.40 ± 0.38	60.70 ± 0.88	76.80 ± 0.42
II (0.2 g of C)	0.00	20.60 ± 0.35	37.30 ± 0.66	57.60 ± 0.49	73.20 ± 1.26	87.50 ± 1.51
III (0.2 g of B)	0.00	22.30 ± 0.38	37.50 ± 0.65	62.51 ± 1.07	78.60 ± 1.36	94.70 ± 1.64
IV (0.2g of P.)	0.00	17.00 ± 0.28	25.00 ± 0.43	36.60 ± 0.53	42.90 ± 0.73	55.40 ± 0.94
V(0.2 g of B + P)	0.00	24.10 ± 0.41	36.60 ± 0.61	48.20 ± 0.83	66.10 ± 1.14	83.90 ± 1.45
VI(0.4 g of B +P)	0.00	26.80 ± 0.46	45.50 ± 0.78	57.20 ± 0.56	73.20 ± 1.27	89.30 ± 1.54
VII (2g of B + P+C)	0.00	26.80 ± 0.46	43.70 ± 0.75	79.50 ± 1.38	92.92 ± 1.61	100.00 ± 0.00

Note: N = Normal saline, B= Bovine mucin, C= Cicatrin®, P= Prosopis gum

to have reduced the colony of the organisms formed with days of treatment that can posed a negative effect on the healing rate.[11] The excellent healing effect when the three were combined as seen in group VII could be attributed majorly to the effect of mucin and cicatrin®, although the bioadhesive property of prosopis gum can not be ignored as this help in adhered samples to the wound surfaces when the combination was applied to allowed long contact time. Healing in this untreated group I, positive control may be due to it self immunity which is natural in a healing process. It is important to note that throughout the period of wound treatment, the samples did not cause any form of irritation or pain to the animals as the rats neither show any signs of restlessness or scratching or biting of wound site when the samples were placed on the wound sites.

CONCLUSION

The mucin from bovine has wound healing activity compare to prosopis,or Cicatrin®. Admixture of bovine mucin, prosopis gum and cicatrin® powder resulted in a better action than only cicatrin. It can be encourage using combination of mucin, prosopis and cicatrin than use of mucin alone in treatment of wound, as well as cicatrin®. Mucin mechanism of action in wound healing is not yet understood.

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