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Full Length Research Paper

Application of snail mucin dispersed in detarium gum gel in wound healing

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This study is aimed at describing the effects of snail mucin dispersed in detarium gum gel on wound healing in rats. The gels were prepared from a mixture of snail mucin and honey and the detraium gum gel. A similar gel containing only the mucin was also formulated. It was observed that the gels containing both the snail mucin and the honey had greater healing effect than the gel containing the mucin alone. Complete wound healing was observed on day 13 in all the cases studied with mucin and honey combinations and over 80 percent healing was achieved for all the cases with mucin alone dispersed in the gum gel.

Key words: Snail mucin, honey, detarium gum, gel, wound healing.

INTRODUCTION

Many factors affect wound healing. Bacteria infection is one of the major factors that affect wound healing. The invasion of bacteria directly to the wound produces inflammation and fluid exudation which interferes with healing. In addition, bacteria toxins cause tissue damage and delays fibroplasias as well as collagen synthesis (Obaseki-Ebor et al., 1983).

Deficiency resulting from nutritional constituents' especially protein, vitamin A, B and C also delays wound healing (Hunt et al., 1969). Protein is essential for body repairs through the reaction of its constituent amino acids. Building and repairing issue require adequate amounts of calories and protein to fuel the repair mechanisms, as the skin and underlying tissues are made of protein. Vitamin A stimulates fibroplasias and may be especially useful in a topical preparation for skin injuries in people taking corticosteroids. Vitamin C is needed to make collagen (connective tissue) that strengthens skin, muscles, and blood vessel and to ensure proper wound healing. Severe injury appears to increase vitamin C requirements and vitamin C deficiency causes delayed healing. Topical application of vitamin E is sometimes recommended for preventing or treating post-injury scars (Levine, 1986).

Some drugs affect wound healing; for example, long term, high doses of corticosteroids (Jenkins et al., 1986). A topical preparation of chamomile combined with corticosteroids and antihistamines has been used to speed-up wound healing in elderly people with stasis ulcers caused by inadequate circulation (Jenkins et al., 1986). Horse chestnut contains a compound called aescin that acts as an anti-inflammatory and reduces oedema following trauma (Guillaume and Padioleau, 1994).

It is important that wounds be properly cleaned and dressed before any preparations are applied. This prevents infection. Bacterial appear to contaminate all surgical wound, but not all wound may be affected. Infection of surgical wounds depends on the bacterial inoculums, the virulence of the bacteria and the duration after contamination. Apart from the bacterial factors, is the impaired host defense due to old age, poor physical state, malnutrition and systemic diseases. Necrotic tissue and reduced blood supply to the wound are other factors (Romito, 1995; Roberts et al., 1998). Since bacterial growth flourishes in dead tissues, the presence of dead or devitalized tissues in a wound is an invitation to infection. Where dead space is present fluid accumulates and serves as a culture media for bacterial growth. In addition, fluid accumulation within the tissue limits migration of reparative cells. In spite of improved aseptic techniques to avoid wound contamination as well as the use of antimicrobial agents, wound contamination and infection remain a post operative complication which could lead to even post-surgical death.

If animal skin integrity is compromised by accidental or surgery trauma, infectious agents have access into the wound to cause contamination and infection of the soft tissue locally. The infectious agents can as well get entra-

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nce into the blood stream which carries it to distant organs to set up other foci of infection.

The sources of contamination and infection are the skin of the patient, the surgical equipment, theatre (hospital environment), patient, the surgeon and the type of surgery performed (Forest, 1982).

For around 2000 years, honey has been used to treat a variety of ailments through topical application. Modern research into the use of honey as an antimicrobial agent has revealed its potential for treating a variety of ailments. Anti-bacterial properties of honey are the result of the low water activity causing osmosis, hydrogen peroxide effect and high acidity (Cooper et al., 1999; Khristov and Mladenov, 1961).

Honey is a poor environment for the growth of organisms since it has a low water activity. Additionally, the use of honey reduces odours, reduces swelling and reduces scarring; it also prevents the dressing from sticking to the healing wound. It acts as an antiseptic/antibacterial agent. It is an excellent natural preservative. In ancient history, the Ancient Egyptian and Middle-Eastern people also used honey for embalming the dead. However, only rich and powerful people had the luxury of this type of funeral.

The pH of honey is commonly between 4 –5. This relatively acidic pH level prevents the growth of many bacteria responsible for infections (Khristov and Mladenov, 1961; Adikwu and Ndu, 2006).

Mucins are a family of large glycosylated proteins (50 %w/w carbohydrate). Mucins are group of nitrogenous substances secreted by a mucous gland. Although some mucins are membrane-bound due to the presence of a hydrophobic membrane – spanning domain that favours retention in the plasma-membrane, the concentration here is on those mucins that are secreted on mucosal surfaces and saliva. Mucin protein backbones are characterized by numerous tandem repeats that contain proline and are high in serine asparagine, hydroxylysine and/or threonine residues (Adikwu, 2006). The structures occur in many life forms, and are prevalent and important in mammalian tissues.

The attached carbohydrate may have several effects; it may help the protein to fold in the proper geometry, stabilize the protein, attract physical properties such as solubility or viscosity, help it to orient correctly in membranes or, make it recognizable to another biochemical or cell. Many proteins released by cells to the blood and other fluids are glycoproteins.

Mucins are secreted as massive aggregate of proteins with molecular masses of roughly 1 to 10 million Da. Within these aggregates, monomers are linked to one another mostly by non-covalent interactions, although intermolecular disulfide bonds may also play a role in this process. They are mainly secreted in the intestine but also in airways and other body membranes (Adikwu et al., 2005).

Snails produce copious mucin which is often referred to as slime. The wound healing property of snail mucin has been reported (Adikwu and Ikejiuba, 2005). Similarly, its physiological and toxicological properties have been documented (Adikwu and Nnamani, 2005). The use of the mucin in a muco-adhesive gel preparation and when fortified with honey and its effect on wound is reported in this work.

EXPERIMENTAL

Materials

Acetone (BDH Chemicals); Diazepam injection (GlaxoWellcome), methylated spirit (Hardis and Dromedras) and distilled water were from an all glass still. The mucin was obtained from a batch prepared in our laboratory following earlier established procedures (Adikwu et al., 2005). Purified honey was obtained from the local market and diluted with sterile, distilled water to obtain a viscosity grade that was equivalent to that stated in the Pharmaceutical Codex (The Pharmaceutical Codex, 1979).

Preparation of detarium gum

The seeds of the plant were fried in hot air oven, soaked for 12 h, peeled and milled into small particles. The milled particles were soaked for 12 h and sieved with muslin cloth, precipitated with acetone and left under room temperature for 3 h. The precipitate was collected on a Buchner funnel by means of pressure from a vacuum pump. It was placed in a vacuum desiccator for 4 days until dried. The dried material was then milled into smaller particles and was sieved with 250 µm sieve and the fine particles were collected into a clean, amber-coloured bottle and stored in a cool condition until used. This procedure is in accordance with an earlier reported one (Chukwu, 1992; Ozumba and Bandgudu, 1992).

Formulation of the gels

A 1.5 g quantity of fine particle of detarium gum was weighed into 8 different clean beakers and 50 ml of distilled water was added into each of the 8 beaker and vigorously shaken until uniformly dispersed. Mucin which ranged from 200, 400, 800, to 1000 mg, was added in appropriate beakers and stirred for 5 min, and the gelation was allowed to further take place for three hours undisturbed. For the formulations containing the honey, the required quantity of the honey was added to the mixed gum gel containing the mucin and stirred gently again, continuously until a homogenous production was obtained (Table 1). Each product was placed in a clean, wide-mouthed bottle and labeled. The preparations were all stored in refrigerated environment until used.

The formulae above were in the production of the various gels. Each gel was prepared basically with variations of the materials as indicated in the table except for detarium gum which was kept constant (Table 2).

Animals

Sixteen male healthy albino rats obtained from Animal Laboratory of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka, were used in the study. The animals were quarantined for a period of one week to ensure stabilization before use.

Table 1. Quantities of the ingredients used in the formulation of the gels.

Batch	Detarium microcarpum (G)	Mucin (MG)	Honey (MG)	Water (ML)
1.5 g D.M.G + 200 mg Mucin + 200 mg Honey	1.5	200	200	50
1.5 g D.M.G + 400 mg Mucin + 400 mg Honey	1.5	400	400	50
1.5 g D.M.G + 800 mg Mucin + 800 mg Honey	1.5	800	800	50
1.5 g D.M.G + 1000 mg Mucin + 1000 mg Honey	1.5	1000	1000	50
1.5 g D.M.G + 200 mg Mucin	1.5	200	-	50
1.5 g D.M.G + 400 mg Mucin	1.5	400	-	50
1.5 g D.M.G + 800 mg Mucin	1.5	800	-	50
1.5 g D.M.G + 1000 mg Mucin	1.5	1000	-	50

Table 2. Effect of various gel preparations of mucin and honey on wound size of the rats.

Batch	Mean wound size (MM) on days post surgery ± S.EM				
	1ST	4th	7TH	10TH	13TH
1.5 g D.M.G +200 mg Mucin + 200 mg Honey	20±3.64	16±3.04	11.5±1.65	6.3 ±2.11	0
1.5 g D.M.G + 400 mg Mucin + 400 mg Honey	20±2.88	13.5±6.48	11.5±1.05	0	0
1.5 g D.M.G + 800 mg Mucin + 800 mg Honey	20±2.88	13±2.27	9±1.29	0±0.00	0
1.5 g D.M.G + 1000 mg Mucin + 1000 mg Honey	20±2.88	10.5±2.22	7.5±1.06	0.00	0
1.5 g D.M.G + 200 mg Mucin	20±2.88	14.5±2.08	12±1.69	7.5±0.46	2.5±0.69
1.5 g D.M.G + 400 mg Mucin	20±2.88	13±1.87	9.5±1.77	4.5±0.41	1.5±0.08
1.5 g D.M.G + 800 mg Mucin	20±2.88	9±1.30	3.3±0.41	1.3±0.16	0
1.5 g D.M.G + 1000 mg Mucin	20. 0±0	7.5±1.72	2.0±1.15	0	0

Feeds consist of grower's marsh and water was provided for the albino rats. The weights range from 247 – 358 g.

Preparation of wound site in experimental animals

The wound site was prepared following the incision wound model (Glowania et al., 1987). The albino rats were anaesthetized with diazepam (0.2 mg/kg body weight) and the hairs on the skin of the animals' back were shaved with a sterilized razor blade. A circle of diameter 20 mm was marked on each right side of the thigh of the animal's skin surface, and the skin dissected out. The area was measured immediately by tracing out the wound area using a transparent tracing paper and the squares counted and the area recorded.

Determination of the rate of wound healing

Treatment was initiated immediately after the incision was made by applying the gel on the wound and then once in every two days. All the gels were applied topically using sterile cotton wool. The wound area of each animal was measured while the animals were under diazepam anesthesia on the days following post surgery. Each application was evaluated in 4 rats per group and the results shown are a mean of 4 determinations. All the procedures followed World Health Organization (WHO) Procedures for Biomedical Research Involving Animal Subjects, 1982.

RESULTS AND DISCUSSION

There was a general and progressive decrease in wound radius of the animals with time (Table 3). By the 13th day the wound area of all the animals treated with a mixture of the mucin and honey in the gels had rescinded.

The combination of the mucin and the honey in the gum gel produced better wound healing than when the mucin was used alone. Healing of wound is a complex phenomenon involving various phases, coagulation, inflamemation, collagenation, wound contraction, epithalialization and remodeling. The phases between coagulation and collagenation are intimately related while that of wound contraction and epitheliazation is independent of each other but run concurrently (Prudden and Allen, 1965). The process of wound healing may be aided by the use of adhesive materials such as the detarium gel since it enables intimate contact of the healing materials with the wound surface. Moreover, it has been observed that honey and snail mucin have adequate wound healing properties (Cooper et al., 1999; Adikwu and Ikejiuba, 2005). Honey when used for wound healing produces little or no side effect. Snail mucin has also been reported to heal wounds (Adikwu and Ikejiuba, 2005). It was observed in this study that snail mucin promotes regeneration of hairs when combined with honey in the animal experiment.

Snail mucin played an important role in wound healing as it enhances the skin natural regenerative response on the formation of new tissues, probably through immune response. The smoothness observed in the healing may indicate that the preparation may prevent kelloid formation.

Table 3. Percentage size reduction of the wounds.

Batch	Wound size reduction (%)			
	4^{TH} Day	7^{TH} Day	10^{TH} Day	13^{TH} Day
1.5 g D.M.G +200 mg Mucin + 200 mg Honey	20±3.64	47.5±7.91	68.5	100
1.5 g D.M.G + 400 mg Mucin + 400 mg Honey	32.5±4.67	47.5±9.61	100	100
1.5 g D.M.G + 800 mg Mucin + *00 mg Honey	52.5±3.95	62.5±8.94	100	100
1.5 g D.M.G + 1000 mg Mucin + 1000 mg Honey	50±3.18	72.5±6.09	92.5±5	100
1.5 g D.M.G + 200 mg Mucin	30.8±4.43	40.0±5.51	62.5±4.11	87.5 ± 6.82
1.5 g D.M.G + 400 mg Mucin	35.0±5.04	57.5±5.04	77.5±2.00	92.5± 3.41
1.5 g D.M.G + 800 mg Mucin	55.0±7.92	82.5±7.11	95.35±2.00	100
1.5 g D.M.G + 1000 mg Mucin	62.5±8.80	90±7.48	100.35±4.79	100

Honey has been shown to have several advantages in healing of wound. It is known to prevent infections and reduce the inflammation, swelling, pain and foul-smell of wounds rapidly. It is reported to induce sloughing of necrotic tissue, thereby hastening granulation and epithelialization. Wound is regarded as healed if there is a restoration of the wounded or inflamed tissue to normal condition. Wound healing processes are generally classified into:

a) Healing by first intention
b) Healing by second intention; and
c) Healing by third intention; depending on the nature of the edges of the healed wounds (Mazzotta, 1994).

When mucin is combined with honey, and applied to a clear incised wound, healing could be said to occur by first intention., that is, the healing occurs by a process that closes the wound edges with little or no inflammatory reaction, and in such manner that no scar is left. The aim of modern surgery is to effect healing by first intention with little post surgical tissue necrosis, as wound edges are united firmly, granulation tissue are not visible and hence the scar is minimal. No suppuration was noticed throughout the treatment and healing period when mucin was used alone and in combination with honey.

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