NOVEL CHITOSAN-HONEY OINTMENT FOR CONTROLLED RELEASE IN WOUND HEALING AND SKIN BURN

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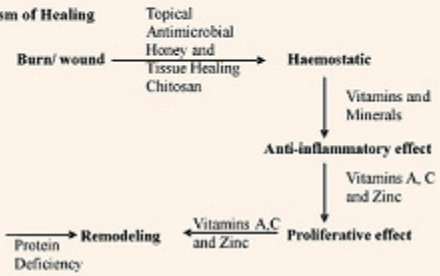
Introduction

Bacteria is the main cause for burn and wound not healing fast enough and with the recent outbreaks of antibiotic-resistant strains of bacteria, this is becoming more of a problem. Honey acts mainly as a hyperosmolar medium and prevents bacterial growth. Because of its high viscosity, it forms a physical barrier, and the presence of enzyme catalase gives honey an antioxidant property. The antibacterial activity of honey is mainly due to inhibines in honey. These inhibines consists of hydrogen peroxide, flavinoids, and phenolic acids, plus many other unidentified substances. A number of reasons for this have been suggested: shrinkage disruption of the bacterial cell wall due to the osmotic effect of the sugar content, induction of an unfavorable environment with low-water activity, thereby inhibiting bacterial growth, a low pH of 3.6 and the fermentation of honey producing alcohol.[1] Chitosan composes of glucosamine and N-acetylglucosamine, which are constituents of mammalian tissues. It is a non-toxic, biocompatible, and biodegradable polymer. Chitosan has good adherence which reduces pain, facilitates decontamination, prevents peripheral channeling into the wound by bacteria, and promotes bonding to tissues. The chitosan dressing must be an absolute barrier to bacterial ingress and could prevent egress of wound organisms to the surface of dressing.[2]

Although many previous studies with the aim of studying the effectiveness of honey and chitosan individually in the treatment and control of both acute and chronic wounds and burn in both human and animals have been undertaken, there is as yet no evidence of their natural combination usefulness in the treatment of burn therapy.

Machanism of Healing



Sterilization of honey by means of ozonization

Honey was ozonized in order to obtain a honey that comprises substantially no fungi and yeasts and with a maximum of 100 colony-forming units per gram of viable aerobic bacteria to avoid the risk of wound botulism. Unheated honey was heated to a temperature of at most 40°C in order to make it sufficiently liquid, such that the ozone gas can come into optimal contact with the honey. In a specially preferred embodiment of a method according to the invention, the honey is ozonized with an ozone generator that produces 120 g/hour (24 g/Nm^3)-of ozone at a flow rate of 15 l/min and an O_2 pressure of 1 bar.[3]

Method of preparation

The ointments were prepared by admixing chitosan in a sigma blade mixer with methyl cellulose and with/without penetration enhancer (diisopropyl adipate). Honey was then mixed into the sigma blade mixer with the solution of chitosan and methyl cellulose. Finally, glycerol was added under continued stirring. The ointment obtained would have highly viscous consistency.

The ointments containing vit. A, C and E were first solubilized in ethanol.

Composition of ointments

Ingredients	F0	F1	F2	F3	F4	F5	F6	F7	F8	F9
Chitosan (% w/w)		35	35	35	35	35	35	35	35	35
Methyl cellulose (% w/w)		30	30	30	30	30	30	30	30	30
Diisopropyl adipate (% w/w)			3	3	3	3	3	3	3	3
Honey (% w/w)	30	30	30	30	30	30	30	30	30	30
Glycerol (% w/w)		5	2	1.7	1.5	1.8	1.2	1.3	1.5	1
Retinyl palmiate (% w/w)				0.3			0.3		0.3	0.3
Ascorbyl palmitate (% w/w)					0.5		0.5	0.5		0.5
Tocopheryl acetate (% w/w)						0.2		0.2	0.2	0.2

Characterization of ointments

Appearence : Visual inspection
Consistency or hardness: Penetrometer
Spreadability: Mutimer Apparatus
Tube Extrudability: Wood Apparatus
Accelerated Stability studies: As per ICH guidelines

Irritancy test

Dorsal hairs at the back of the rats were clipped off one day prior to the commencement of the study. Animals showing normal skin texture were housed individually in cages with copography meshes to avoid contact with the bedding. One side of each animal was used for intact study and the other for testing on abraded skin. Abrasion was non-bleeding incision of stratum corneum. About 50 mg of test sample was applied over one square centimeter area of intact and abraded skin. Animals were immobilized in a restrainer for 24 h. Skin responses were evaluated according to the visual analog scale used in the Draize technique.

Efficacy study

Fifty-four male mice underwent skin excision (10x10 mm) from the nape of the neck, to the depth immediately above the first layer of muscle, in a study by Bergman et al (1983).

Deep skin burns were applied in twelve places on the flanks of pigs in a study by Postmes et al (1996) to study the efficacy of chitosan-honey ointment.

Antimicrobial study

Five species of bacteria *Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, and Klebsiella pneumonia* were collected. Bacteri were isolated from the infected burns and identified by standard methods.

The antibacterial activity of all honeys formulations was assayed by the Hole-Plate Diffusion Method.

Controlled release antibacterial activity

The antibacterial activity of honey formulations was mainly ascribed to the production of hydrogen peroxide.

Results and discussion

Characterization of ointments

Parameters	F1	F2	F3	F4	F5	F6	F7	F8	F9
Appearance	Brownish Yellow	Brownish Yellow	Brownish Yellow	Brownish Yellow	Brownish Yellow	Brownish Yellow	Brownish Yellow	Brownish Yellow	Brownish Yellow
Consistency	226	225	224	225	225	224	224	224	225
Spreadability g.cm/sec	6.22	6.23	6.23	6.24	6.24	6.25	6.25	6.27	6.34
Extrudability (%)	76.8	76.1	76.2	76.2	76.2	76.2	76.2	76.2	76.8
Stability test	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed	Passed

From the results, it is clearly evident that all formulations showed good extrudability, homogeneity and spreadability. There was no significant effect of penetration enhancer, Vit. A, C and E on the appearence, consistency, spreadability, extrusibility and stability studies.

Irritancy study

Test for irritation resulted in selecting the ointments for further tests because it proved to be non-irritant.

Efficacy study

It was observed that diisopropyl adipate the increase the penetration of ointment formulations through the skin have more efficacy plays the important roles. The Formulation (F9) tissue underwent more rapid and more extensive epithelisation than other formulations since vitamins A, C, E and penetration enhancer were present.

Antimicrobial study

Species	Honey- formulation zone of growth inhibition		
	0	1:2	1:4
Staphylococcus aureus	33	15	10
Escherichia coli	31	18	12
Proteus mirabilis	26	15	12
Pseudomonas aeruginosa	24	15	11
Klebsiella pneumonia	21	13	10

The undiluted honey-formulations recorded 96.6 % antibacterial activity, followed by 1: 2 and 1: 4 diluted formulations. The ability of honey to kill microorganisms has been attributed to its high content of tetracycline derivatives, peroxidase, fatty acids, phenols, sugars and amylase. Chitosan improves substrate supply in local environment promoting epithelization and angeiogenesis.

Controlled release antibacterial activity

The controlled release of honey from chitosan formulation showed that continual presence of low concentrations of hydrogen peroxides kills bacteria more effectively than single high dosage treatment and fibroblast are not damaged.

Conclusion

Therefore chitosan-honey ointment very effectively treats wound and burn by slow release of small amounts of hydrogen peroxides, without damaging the regenerating tissue.

References

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