Research Article

Available online through
www.ijrap.net

IJRAP
ISSN 2229-3566

DEVELOPMENT AND *IN VITRO* EVALUATION OF CHITOSAN GEL FOR WOUND HEALING ACTIVITY

Shinde Anilkumar J., Khade Kishorkumar M*, Kadam Atul R., More Harinath N
Department of Pharmaceutics, Bharati Vidyapeeth College of Pharmacy, Kolhapur-413016, (MS), India

Received on: 03/01/2011 Revised on: 01/02/2011 Accepted on: 15/02/2011

ABSTRACT
The present study aims to evaluate the wound healing activity of gel containing chitosan in rats. Chitosan is reported for wound healing activity, four optimized gel formulations were prepared, out of that CG-4 showed promising physical properties like colour, pH, consistancy, spreadability, extrudability as that of marketed wound healing cream formulations. Topical application of the test formulation CG-4 gel formulation was used for treatment group, which has showed significant wound healing activity in excision wound model. Percentage wound closure, period of complete epithelisation and scar size reduction on complete epithelisation showed P value<0.001 as comparable to marketed formulation (1%w/w). Dead space wound studies also showed significant increase in Hydroxyproline content indicating promotion of collagen formation and ultimately wound healing activity. The present study thus offers a valuable insight into the claimed wound healing potential of the test formulation.
KEYWORDS: Chitosan, gel, wound healing, wound closure, Dead space wound.

***Corresponding Author**
Kishorkumar Khade, B. Pharm Final year Student, Bharati Vidhyapeeth College of Pharmacy, Kolhapur.416013.Maharashtra, India

INTRODUCTION

Chitosan, natural polysaccharides is comprising copolymers of glucosamine and N- acetylglucosamine and can be derived by partial deacetylation of Chitin from crustacean shells.[1] Chitin is the second most abundant natural polysaccharides after cellulose. It's sugar backbone consist of β1 4-linked glucosamine with a high degree of N- acetylation, a structure very similar to that of the cellulose, the only difference being replacement of the hydroxyl moieties by amino groups. Chitosan is reported for variety antiseptic, antibacterial, and wound healing etc.[2]

Wound is a distrupted state of tissues caused by physical, chemical, microbial or immunological etc., ultimately healing either by fibroplasias. Healing processes through three general stages inflammatory, proliferate, repair and remolding.[3,4] During inflammatory stage, as a result of injury, the blood circulation in the local area is reduced, which leads to hypoxia, acidosis and low pH. Naturally the investigative curiosity to promote wound healing continued. Many natural or ayurvedic preparations claims to be useful in wound healing. However, very few investigations have been made to assess the efficacy of it. No attempt is made to formulate chitosan gel and observed wound healing property. Hence we have tried to formulate a chitosan gel and evaluate it for various physical parameters and wound healing property.

MATERIALS AND METHODS

The gift sample of Chitosan powder from (Central Institute of Fishries Technology, Kochi), Acetic acid, Glycerine (S.D. Fine chem.,Mumbai) and Sodium hydroxide (loba chemie Pvt Ltd, Mumbai). Hydroxyproline, Methyl red, Conc.Hydrchloric acid, Sodium hydroxide pellets, Chloramine-T, P-dimethylamino benzyldehyde, citric acid monohydrate, glacial acetic acid, sodium acetate trihydrate, Toluene, methylcellulose & Perchloric acid obtained from loba chemie Mumbai. All remaining chemicals were of AR grade.

Preparation of Chitosan Gel formulation

Gel formulation composition stated in **table 1**. The optimized Gel so formed were called as CG-1, CG -2, CG -3 and CG -4.

Evaluation of Chitosan Gel

All formulations CG -1, CG -2, CG -3 & CG -4 along with two marketed preparations A & B were subjected for preliminary evaluation.

pH

pH meter was standardized as per manufacturer's manual. 5% solution of Gel in distilled water was prepared and determined pH.

Viscosity

Viscosity is an important parameter to be studied in semisolid evaluation. All test and marketed formulations were tested for viscosity by using Brookfield Viscometer.

Spreadability

Spreadability of all test and marketed formulations was measured by using 'spreadability apparatus' After applying weight, the time in seconds, required to separate the slides was noted. Spreadability of each formulation is reported in seconds.

Extrudability

Extrudability apparatus' was used to determine the Extrudability of gel. A closed Collapsible tube containing gel was pressed firmly at the crimped end. When the cap was removed, gel extrudes until pressure dissipated. Weight in grams required to extrude 0.5 cm ribbon of gel in 10 seconds was determined. The average extrusion pressure in grams is reported.

Stability

The gel formulations under test and marketed were filled into collapsible tubes and properly capped tubes were subjected for stability studies at 4°C & 37°C for eight weeks. The gels were observed after each week for possible changes in colour, odor, consistency, pH, Viscosity, spreadability, Extrudability and stability of Gels are reported in **table 2**.

Animal selection and Preparation

Healthy adult albino rats weighing 150-250 gm of either sex were used for study. The albino rats were 4 to 5 months old and were divided in to two groups each group containing 6 adult albino rats. The groups were named as (A), (B). Group (A) being control, Group (B) treatment group treated with Gel formulation. The animals were housed individually with food and water given ad libitum. Animals were depilated on dorsal side and were starved 12 hrs prior to wounding. The 'excision and dead space wound model' was used for study.[5]

Excision wound model was employed to study the rate of wound contraction, epithelisation, scar size and percentage period of complete epithelisation [6,7]. A round seal of 2 cm diameter was impressed on the hair removed dorsal throracic central region of the chloroform anaesthetized rats. Full thickness skin from the demarked area was incised to produce wound. The wound was washed with cotton swab soaked in saline. The animals were maintained in separate cages.[8,9]

Dead space wound model,[10] was employed to assess the extent of collagenation. Animal groups were as that of excision wound. Under chloroform anesthesia subcutaneous dead space wounds were inflicted in the region of axilla and groin, by making a pouch through a small nick in skin. Implanting sterile cotton pellets weighing 10 mg by the technique of 'Darcy' et.al induced granuloma formation as described by Turner.[11] 1ml/kg body weight dose was given orally per day. The granuloma tissues were removed on 10th day. Cotton pellets granuloma was excised from dead space wounds and were dried over night at 60ºc. Their weights were noted and expressed as mg/100 gm body weight. The tissues so obtained were stained with haematoxylin and eosin to assess fibroblast population, infiltrating cells, hydroxylproline content and thickness of the tissue.

Wound closure

The experimental protocols were approved by the institute animal's ethics committee at Bharati vidyapeeth, college of Pharmacy, Kolhapur. In animals belonging to groups A & B every day the excision wounds were cleaned with cotton swab dipped in normal saline solution. For control group (A) wounds were not applied with anything. For Treatment group (B) was applied locally ones a day to full area of wound. The formulated gel was applied on the wound once daily for 15 days starting from the day of wounding. Wound contraction 4th, 8th, 12 th of wounding day and period of epithelisation, and scar area were measured.[12] Wound contraction was studied by tracing raw wound area on polyethylene paper every 4 days till wounds were completely covered with epithelium.[13] These wound tracing were retraced on a millimeter scale graph paper, the wound area determined and expressed as percentage of original wound size.[14] The data was presented in **Table 3**. The collagen content in granulation tissue was observed on10th post wounding day histopathologically reported in **table 4**.

RESULTS AND DISCUSSION

There was need to development of Chitosan Gel formulation and evaluation for it's wound healing activity. Preliminary characterization of Gel has shown that pH of all formulations was approaching towards neutrality. Amongst the test formulations CG-1, CG -2, CG -3 and CG -4, the Viscosity, Spreadability and Extrudability of CG -4 was quite closest to marketed formulations A and B. Hence formulation CG -4 was used for wound healing studies. All test and marketed formulations did not show any sign of change in colour, odor and consistency.

Excision type of wound healing showed that, percentage closure of wound in treatment group was 94.53% within

12.5 days and 18.50 days were required for control group for complete epithelisation to take place and Scar Size 21.14 sq.mm indicated that the least scar area was found for formulation CG -4 applied wounds, when compared with controlled groups. This signifies better wound healing promotional activity. Histological study performed for excision wound showed increase in the collagen, fibroblasts on the 4th post wounding day, which concludes the promotion in the process of healing.

Dead space wound studies from control group has shown predominant acute inflammatory exudates and very negligible amount of collagen. Treated group shows few fibroblasts, some are area of acute inflammatory exudates and some amount of collagen. Hydroxyproline is an amino acid present in the collagen fibers of granuloma tissue. It was found to be 1.46 mg/gm in 10 days old granuloma tissues. Lastly the group which was treated with CG- 4 formulation showed significant amount of thick wavy bundles of collagen without exudates.

CONCLUSION

Excision type of wound healing showed promising results for percentage wound closure, reduction in scar size on complete epithelisation, time for complete epithelisation and observation of fibroblasts, cellular elements and collagen fibers. The difference between the mean percentage closures in wound area of drug treated animals as compared to control was statistically significant. It was observed that there is significant increase in wound closure on 12.5 day. The formulation CG-4 has statistically significant wound healing activity p=0.001 in healthy adult albino rats. There is a need to develop formulations, which are responsible for wound healing action. This will facilitate their formulation in to a better dosage form for promotion of wound healing activity.

ACKNOWLEDGEMENT

We are grateful to the Central Institute of Fishries Technology, Kochi for gift sample of Chitosan powder, and the Principal Dr. H. N. More Bharati Vidyapeeth College of Pharmacy Kolhapur for providing excellent facility to carry out this work.

REFERENCES

1. Paul W, Sharma CP. Chitosan is a drug carrier for the 21st century., a review, S.T.P, Pharm. Sci., 2000;10: 5- 10
2. Illum L, Chitosan and it's use as a pharmaceutical excipient, Pharm Res. 1998;15:1326 -1331.
3. Bairy KL & Mallikarjun Rao, Wound healing profiles of orally administered Zinc gluconate, The Antiseptic 1993; 90 (4):171-173.
4. Hunt TK and Waltone Van, Winkle Jr., Normal repair in fundamentals of wound management 1979; 2:67.
5. Ramirez A, Soroff HS. Experimental wound healing., Surg. Gynecol. Obstet**.** 1967;124: 369-386.
6. Oessner JR. The determination of hydroxyproline sample containing small proportion of amino acids. Arch. Biochem. 1961;93:440
7. Patil PA and Kulkarni DR. Effect of antiproliferative agents on healing of dead space wounds in rats., Ind. J. Med. Res. 1984;79: 445-447.
8. Litchfield JT, Wileoxon FA, J. Pharmacol. Exp. Ther. 1949; 95: 99-113
9. Rao CM, Ramesh KV, Bairy KL, Dr. Kulkarni. Ind. J. Exp. Biol**.** 1991;29:156-158.
10. Ehrlich HP and Hunt TK. Effect of Cortisone and Vitamin A on Wound healing. Ann.Surg**.** 1968;167:324.
11. Oessner JR. The determination of hydroxyproline sample containing small proportion of amino acids., Arch. Biochem**.** 1961,93:440.
12. Patil MB, Jalalpur SS and Ali Ashraf. Preliminary phytochemical investigation and wound healing activity of the leaves of Argemone Mexicana Linn (Papaveraceae), Indian Drugs 2001; 38(6): 288-293.
13. Jaswanth A, Akilandeswari, Longanathan V, Manimon S. and Ruckmani: Wound healing activity of Aegemarmoes, Indian Journal Pharma Science, 2001; 63(1): 41-44.
14. Shobha S, Nandig and Rao S Gurumadhava: Effect of phytopharmaceutical formulation- an indigenous formulation on wound healing in rats, Indian Drugs 2000; 37(9): 417-420.
15. Bairy KL, Jacob AP, Effect of tolmez and its copper complex on wound healing, Indian Journal of Experimental Biology, 1995; 33:201.

Table 1: Formulations of Chitosan Gel

Contents	Formulations			
	CG-1	CG -2	CG -3	CG -4
Chitosan Powder	4 gm	3.5 gm	3.7 gm	3.0 gm
Glycerin	6 gm	6.5 gm	6.3 gm	7.0 gm
Glacial Acetic acid in water (1.0%)	q.s	q.s	q.s	q.s
	10 gm	10 gm	10 gm	10 gm

Table 2: Preliminary evaluation of Chitosan Gel

Formulations	pH	Consistency in Centipoise	Spreadability In Seconds	Extrudability in gms
CG-1	6.45	248	4.05	549
CG -2	6.33	235	4.12	544
CG -3	6.47	259	4.14	553
CG -4	6.35	248	2.58	494
A	7.23	239	2.64	496
B	6.28	234	2.21	510

Table 3: Percentage closure of original excision wound area in sq.mm at different time intervals (mean±S.E) in control, Base and Formulation treated groups

Groups (n=5/group)	4th day	8th day	12th day
Control (A) %	25.76 ±1.44	54. 82 ±1.44	65.06 ± 0.74
Treatment with Gel (B) %	56.34 ±1.47[a]	79.82± 0.856 [a]	94.23± 0.78[a]

a=p<.001Vs control

Table 4: Percent Period of complete epithelisation in days, scar size sq mm on complete epithelisation and hydroxyproline content mg/gm of 10 days old tissues, (Mean ± SE)

Groups (n=5/group)	Days	Scar size	Hydroxyproline content
Control (A) %	18.50 ± 0.20	50.01± 1.89	0.986
Treatment with Gel (B)%	12.50±0.25[a]	21.41± 0.54[a]	1.461 ± 0.3

a=p<.001Vs control

Source of support: Nil, Conflict of interest: None Declared