Advances in Bioresearch
Adv. Biores., Vol 7 (6) November 2016: 01-07
©2015 Society of Education, India
Print ISSN 0976-4585; Online ISSN 2277-1573
Journal's URL:http://www.soeagra.com/abr.html
CODEN: ABRDC3
ICV Value 8.21 [2014]

Advances in Bioresearch

ORIGINAL ARTICLE

The Potential Anti-inflammatory and Wound Healing Activities of Chitosan in rats

Saeedan S. Abdulaziz[1*], Gamal A. Gabr[1,2], Gamal A. Soliman[1,3], Mohamed H. Fayed[4] , Mohd Nazam Ansari[1]

[1]Department of Pharmacology, College of Pharmacy, Prince Sattam bin Abdulaziz University, Al-Kharj, KSA.

[2]Agricultural Genetic Engineering Research Institute, Agric. Res. Center, Giza, Egypt.

[3]Department of Pharmacology, College of Veterinary Medicine, Cairo University, Egypt.

[4]Department of Pharmaceutics, College of Pharmacy, Prince Sattam bin Abdulaziz University, Al-Kharj, KSA.

***** E-mail: a.binsaeedan@psau.edu.sa

ABSTRACT

In the current investigation, the anti-inflammatory and wound healing potential of chitosan were investigated. To expose the anti-inflammatory effect of chitosan, we studied its effect against edema induced by carrageenanin rat foot pad at 150 and 300 mg/ kg and compared with the reference, indomethacin (5 mg/kg). The wound healing effect of chitosan was evaluated in rats by excision method and compared with the standard, fucidine cream 2%. The wound-healing activity was assessed by the rate of wound contraction and period of epithelialization. Our results showed that oral administration of chitosan at 150 and 300 mg/ kg to rats reduced carrageenan-induced edema dose-dependently.In assessing wound healing effect, topical application of 5% and 10% chitosan creams accelerated healing of wounds when compared to control.The rate of wound contraction was significantly increased on days 3 - 21in fucidine and chitosan (5 and 10%)-treated animals. The duration of wound epithelialization was decreased in groups treated with the reference standard and chitosan creams than the vehicle-treated group. These results strongly document the beneficial effects of chitosanas anti-inflammatory and for the precipitation of wound healing in rats.

Key words:*Chitosan, Anti-inflammatory, Wound healing, Indomethacin, Fucidine.*

Received 24/02/2016 Accepted 22/05/2016

How to cite this article:
S S. Abdulaziz, G A. Gabr, G A. Soliman, M H. Fayed , M N Ansari. The Potential Anti-inflammatory and Wound Healing Activities of Chitosan in rats. Adv. Biores., Vol 7 [6] November 2016: 01-07**.** DOI: 10.15515/abr.0976-4585.7.6.17

INTRODUCTION

Nautical organisms generate numerous bioactive material, which are having many possible uses. Chitosan is a natural polysaccharide biopolymer derived from chitin, found in the exoskeletons and the cell wall of shrimp, insects and crabs.[1] Chitosan is a natural polysaccharide consisting of copolymer of 2-amino-2-deoxy-D-glucopyranose and b-(1-4)-glycosidic bonds linking N-acetyl-2-amino-2-deoxy-Dglucopyranose (glucosamine).[2]It has some uses in the areas of clarification and purification, paper and textiles, food and nutrition, agriculture, cosmetics, biodegradable membranes and biotechnology.[3]Currently, chitosan is receiving a considerable degree of interest for medical and pharmaceutical uses, as it displays a broaddiversity of biological actions, such as anticancer[4], immune-stimulant[5], anti-allergic [6], hemostatic [7], hypocholesterolemic [8], free radical scavenging [9] and antimicrobial activities[10]. The majorpurposes for this increasing interest are obviously its motivating intrinsic characteristics. Chitosan comprises three kinds of reactive functional groups, an amino/acetamido group in addition to primary and secondary hydroxyl groups at C-2, C-3 and C-6 positions, respectively.[11]Moreover, chitosan is metabolized by particular human enzymes, like lysozyme, and is considered biodegradable. Biodegradation of chitosan liberates non-toxic oligosaccharides as it is being hydrolyzed to ultra-low molecular weight chains that pass through the intestinal barrier and become excreted in

urine.[12]Against this background, the current study was commenced to estimate the potential anti-inflammatory and wound healing effects of chitosan in a rat models.

MATERIALS AND METHODS

Preparation of chitosan

The Fresh shrimp (*Metapenaeus affinis*) shells were collected from a local market, Riyadh, KSA. The shells of shrimp were carefully washed, drained, dried at 60°C for 12 h, and ground to pass through a 250 µm sieve, then stored at -18±2°C till chitin extraction.The dry powders of exoskeleton of the shrimp were treated with HCl, NaOH 1-2 M then with 40% NaOH to extract the chitosan.[13] The degree of deacetylation of chitosan determined by potentiometric titration[14], and the molecular weight was calculated using the value of intrinsic viscosity[15] measured by an Ubbelohde viscometer. Chitosan homogenous solution was prepared using dil. HCl containing 0.010 mol/L which is titrated against 0.1M NaOH.[16]

Experimental Animals.

Wistar male rats weighing 150- 170 g were obtained from the Lab Animal Care Unit,Faculty of Pharmacy, University of Prince Sattam bin Abdulaziz, KSA. The rats were acclimatized to the laboratory environment for one week. All studies were carried out using six rats in each group.

Preparation of chitosan for anti-inflammatory activity

Chitosan was suspended in 3% v/v Tween 80 before oral administration to the experimental animals.

Preparation of chitosan creams for wound healing activity

The topical chitosan creams are prepared according to the formula on Table 1.[17] The petrolatum was melted in a water bath at 70°. The surfactants sorbitan monolaurate and tween 80 were dispersed in the aqueous and oil phases respectively. Quantities of glycerol andchitosan were accordingly mixed together to form the aqueous phase. The aqueous phase was slowly added to the oil phase with continuous stirring at 500 rpm with a Kenwood kitchen mixer (Kenwood, USA). On addition of all the aqueous phase the mixture was mixed for another 5 min before the cream was removed from the water bath and allowed to set.

Table 1: Formula of chitosan creams

Components	Cream base	5% w/w Chitosan	10% w/w Chitosan
Chitosan	-	2.50	5.00
Petrolatum	21.16	21.16	21.16
Glycerol	4.67	4.67	4.67
Sorbitan monolaurate	5.00	5.00	5.00
Tween 80	2.00	2.00	2.00
Water	17.17	14.67	12.17

Acute toxicity study

Acute oral toxicity test was carried out in rats as described by OECD-423 guidelines.[18]Two groups of Wistar albino rats *(n=6)* were fasted overnight. Rats of the 1st group were orally medicated with chitosan at a dose of 3000 mg/kg. The 2nd group(control) received the vehicle (3% v/v Tween 80 in distilled water). Each animal was observed for symptoms of toxicity and/or mortalities for every 15 min in the first 4 h after administration, then every 30 min for the successive 6 h and then daily for the successive 48 h. Since, there was no mortality at this level; the dose of both extracts was increased to 3000 mg/kg and animals were observed for another 48 h.

Doses

Chitosan was safe at the dose of 3000 mg/kg. Thus, doses of 150 and 300 mg/kg that are equal to 1/20 and 1/10 of the highest dose tolerated by rats were selected for the anti-inflammatory study.

Anti-inflammatory activity

The anti-inflammatory activity was estimated in rats using a carrageenan-induced paw edema method.[19]Four groups (n=6) of rats were used. Rats of groups I (normal control) and II (reference) were orally medicated with the vehicle (5 mL/kg) and an aqueous solution of indomethacin (5 mg/kg), respectively. Rats of groups III and IV received 150 and 300 mg/ kg of chitosan suspension in 3% of Tween 80, respectively. One h later, the rats were subcutaneously injected with 100uL of 1% suspension of carrageenan (Sigma chemical co, St. Louis MO, USA) in normal saline into the plantar side of the left hind paw. The paw volume of the rats was estimated directly after carrageenan injection (0 h) and then hourly till 6 h using plethysmograph apparatus. The anti- inflammatory activity was determined as the percentage of reduction of inflammation by using the formula:

% inhibition = 1- (Vt/Vc) X 100.
Where 'Vc' represents edema volume in control and 'Vt' edema volume in groups treated with indomethacin or chitosan.

Wound Healing Activity

Chitosan was evaluated for its wound healing activity in rats using excision wound model. The effect of chitosan on the rate of wound healing was assessed by the rate of wound closure and period of epithelialization.

Grouping of animals

Group 1: Control group (treated topically with cream base, n=6)
Group 2: Reference group (treated topically with 2% fucidine cream, n=6)
Group 3: Treated topically with 5% chitosan cream (n=6)
Group 4: Treated topically with 10% chitosan cream (n=6)

The animals were anaesthetized by intraperitoneal injection of ketamine and xylazine (5 and 2 mg/kg, respectively). Skin of the dorsal area of each rat was shaved using an electrical clipper and disinfected with 70% alcohol. A uniform circular wound of approximately100 mm2 area was excised on the dorsal side of each rat as described by Mughrabi et al.[20] Care was taken to preclude damaging the muscle layer, and the tension of skin was kept constant during the process. The wounding day was considered as day 0. The wounds were treated with the topical application of the vehicle, reference standard and chitosan cream till the complete healing of wounds. The wounds were observed and the area of wounds was measured on 3, 6, 9, 12, 15, 18 and 21 post-wounding day.

Parameters evaluated for wound healing

a. Measurement of wound contraction

The percentage of wound contraction was assessed by tracing the wound on days 0, 3, 6, 9, 12, 15, 18 and 21 after wounding or till the wound gets healed using transparent paper and a permanent marker. The areas of wounds were measured against scale graph paper (mm2). The rates of wound contraction were calculated.[21]

$$\text{Wound contraction (\%)} = \frac{\text{Wound area on day 0} \quad \text{Wound area on day n}}{\text{Wound area on day 0}} \text{X } 100$$

Where n is the number of days: 3^{rd}, 6^{th}, 9^{th}, 12^{th}, 15^{th}, 18^{th} and 21^{st} days.

b. Epithelialization period

The epithelialization period was calculated as the duration per days required for falling of the dead tissue remnants without any residual raw wound.[22]

Statistical analysis

Data analysis was carried out using one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests. $P < 0.05$ was considered statistically significant. Data values are each expressed as the mean ± S.D.

RESULTS

Acute Toxicity Test

Animals exposed to chitosan at doses up to 3000 mg/kg did not show any signs of physical and behavioral toxicity. Chitosan-treated animals did not show restlessness, uncoordinated movements, diarrhea, hematuria, or respiratory distress. Moreover, no mortalities were also recorded within 48h of observation.

Anti-inflammatory Activity

The effect of chitosan and indomethacin in carrageenan induced paw edema in rats is shown in Table 2 and Figure 1. Subcutaneous administration of 100 uL of 1% suspension of carrageenan induced edema in the foot pad of rat hind paw. The maximum volume of the carrageenan-injected foot pad (1.27±0.07 mL) was obtained 3 h after the administration (Table 2).

As expected indomethacin (5 mg/ kg) significantly reduced carrageenan induced paw edema. It inhibited the edema volume by 56.69, 58.55, 59.81 and 56.84% after 3, 4, 5 and 6 h of carrageenan injection, respectively as compared to the control vehicle treated group (Figure 1). Oral pretreatment with chitosan (150 and 300 mg/kg) showed dose-dependent inhibitory activity in carrageenan-induced paw inflammation over a period of 6 h. Chitosan at 300 mg/kg showed the most potent anti-inflammatory effect after 4 h of carrageenan injection with an inhibition rate of 47.74% compared with a 58.55% inhibition with the positive control.

Wound Healing Activity

Table (3) and Figures (2 & 3) show the effect of fucidine and chitosan creams on wound-healing activity in rats inflicted with excision wound. The results of wound healing effects of chitosan showed significant promotion of wound healing activity in the excision wound model. Control group showed least rate of wound healingon the 3rd – 21stday of vehicle application. Topical application of chitosan (5 and 10%) caused a significant concentration related reduction in wound area on the 3rd – 21st day of wounding; compared to the animals of the control group (Table 3).Treatment with standard fucidine 2% also produced significant reduction in the wound area as compared to control animals.

The percentages of wound contraction were 17.48±1.1, 34.56±1.1, 49.17±2.1, 65.22±2.8, 79.42±3.4, 91.35±3.8 and 100±0.0% with chitosan cream5% and 19.63±1.2, 38.07±1.4, 54.50±2.6, 71.54±2.4, 86.57±3.6, 100 ± 0.0and 100±0.0% with chitosan cream10% compared to 10.95±0.9, 21.91±1.3, 33.46±1.2, 43.24±2.5, 53.42±2.1, 64.18±2.7 and 75.14±2.7% in the control group on the 3rd, 6th, 9th, 12th, 15th, 18th and 21stday of treatment, respectively.

Period of wound epithelialization was reduced in groups treated with the reference standard and chitosan creams (5 and 10%) than the vehicle-treated group (Table 3).In control rats, wound takes more than 26 days to heal completely unlike with chitosan cream in which the wounds heal almost completely around days19 and 17 at concentrations of 5 and 10%, respectively.

Table 2: Anti-inflammatory activity of indomethacin (5 mg/kg) and chitosan(150 and 300 mg/kg) against carrageenan-induced paw edema in rats (n=6).

Treatment	Paw volume (mL) after						
	0 h	1 h	2 h	3 h	4 h	5 h	6 h
Control	0.87±0.06	1.04±0.07	1.16±0.07	1.27±0.07	1.11±0.08	1.07±0.05	0.95±0.06
Indomethacin (5 mg/kg)	0.86±0.05	0.73±0.04*	0.61±0.03*	0.55±0.03*	0.46±0.03*	0.43±0.02*	0.41±0.03*
Chitosan (150 mg/kg)	0.88±0.05	0.96±0.06	0.88±0.04*	0.78±0.04*	0.68±0.05*	0.66±0.04*	0.65±0.03*
Chitosan(300 mg/kg)	0.90±0.03	0.87±0.04	0.75±0.04*	0.71±0.04*	0.58±0.05*	0.57±0.02*	0.58±0.02*

*Significantly different from the values of the control rats at P< 0.05.

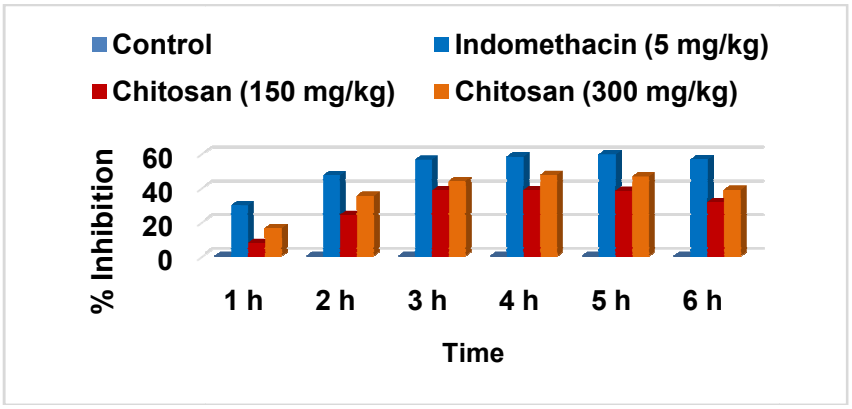

Figure 1: Percentage inhibition of paw edema exhibited by indomethacin (5 mg/kg) and chitosan(150 and 300 mg/kg).

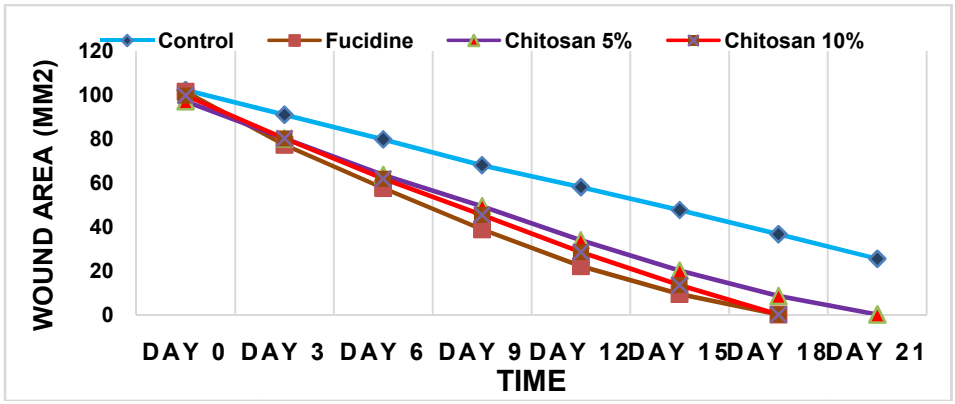

Figure 2: Effect of topical application of fucidine (2%) and chitosan (5&10%)creams on wound area (mm2) in rats (n=6).

Table 3: Effect of topical application of fucidine (2%) and chitosan (5&10%)creams on the percentage of wound contraction and period of epithelization of excision wound model in rats (n=6).

Groups	Wound contraction %							Period of epithelialization (days)
	Day 3	Day 6	Day 9	Day 12	Day 15	Day 18	Day 21	
Control	10.95±0.9	21.91±1.3	33.46±1.2	43.24±2.5	53.42±2.1	64.18±2.7	75.14±2.7	26.4±0.8
Fucidine 2%	23.81±1.2*	43.30±1.7*	61.81±2.5*	78.34±3.7*	90.74±3.5*	100±0.0*	100±0.0*	17.2±0.73*
Chitosan 5%	17.48±1.1*	34.56±1.1*	49.17±2.1*	65.22±2.8*	79.42±3.4*	91.35±3.8*	100±0.0*	19.1±0.77*
Chitosan 10%	19.63±1.2*	38.07±1.4*	54.50±2.6*	71.54±2.4*	86.57±3.6*	100±0.0*	100±0.0*	17.8±0.52*

*Significantly different from the values of the control rats at P< 0.05.

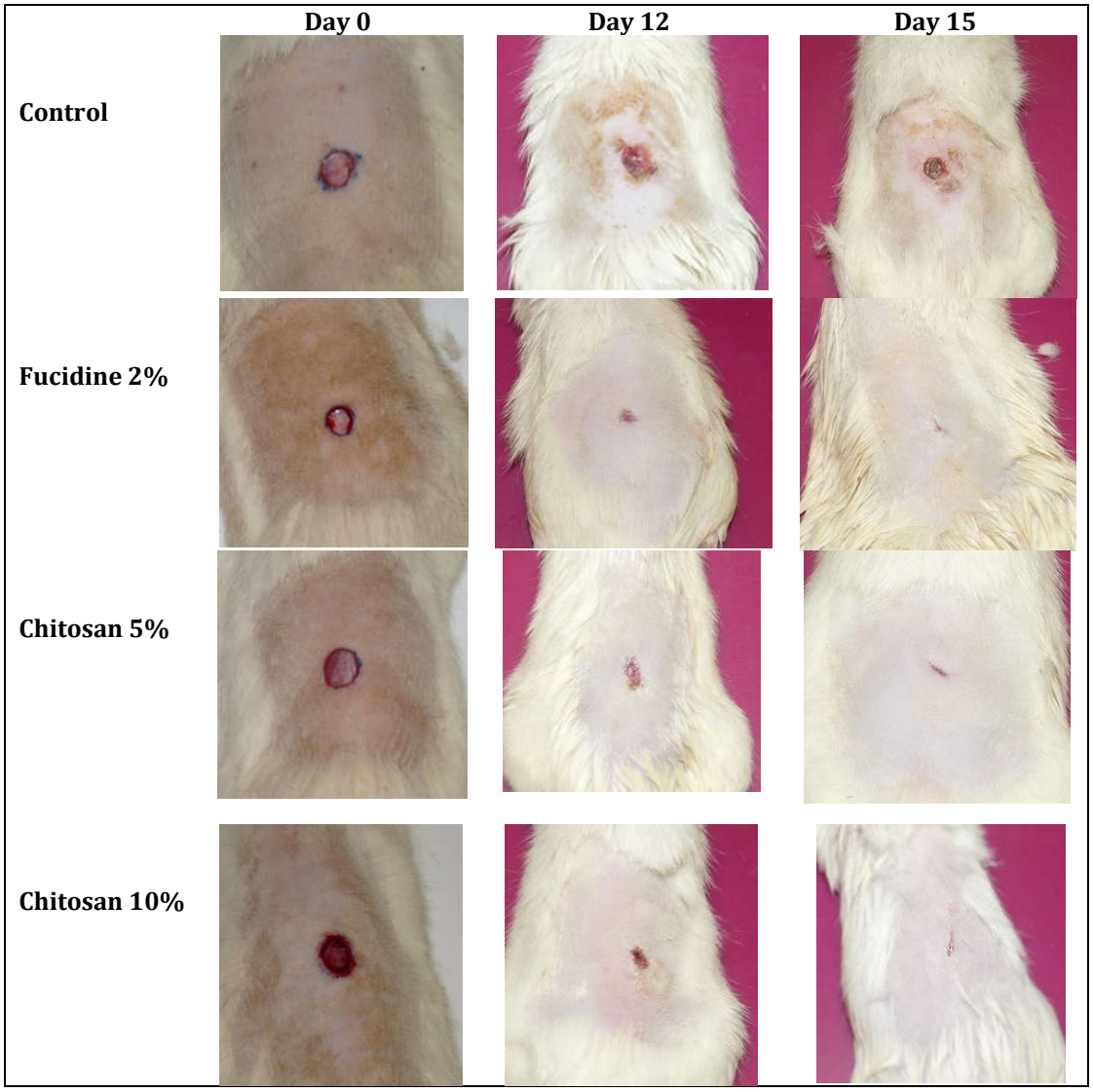

Figure 3: Photographic representation of wound area following topical application of fucidine (2%) and chitosan (5&10%) creams in rats on the days 0, 12, and 15 of treatment.

DISCUSSION

Chitosan was characterized by an elevated degree of safety. Chitosan is a fiber which sticks out to compose a gel in the acid pH of the stomach. When chitosan is matched to common sugars, it was found that it has high degree of safety than these substances.[23]

In this study, oral administration of chitosan at doses up to 3000 mg/ kg did not produce any sign of toxicity and all animals remain alive during 48 h of observation. Therefore, it proposed that oral median lethal doses (LD50) of chitosan were more than 3000 mg/ kg. Accordingly, chitosan can be classified as quietly safe since material having LD50 more than 50 mg/ kg are non-toxic.[24] The absence of apparent

changes may be related to relatively shorter duration of exposure to chitosan which appeared to be safe. In addition, any breakdown of chitosan by colon microflora would release D-glucoseamine which is itself a useful nutrient for human.[1]

In the present study, the anti-inflammatory activity of chitosan has been established in acute model. Carrageenan-induced paw inflammation is a standard assay for acute inflammation that is effectively employed to assess the anti-inflammatory activity of drugs and other compounds.[25]Pretreatment with the cyclooxygenase inhibitor, indomethacin as well as chitosan (150 and 300 mg/kg) exhibited dose-dependent restrained effect in carrageenan-induced paw edema in rats over a period of 6 h. The duration of edema development on carrageenan induced paw edema model in rats is manifested as a biphasic response.[26]The release of histamine or serotonin occurs in the first phase (up to 1 h) whereas the second phase (over 1 h) is associated with the production of bradykinins, prostaglandins, and lysosomes.[26]Chitosan significantly inhibited later phase of carageenan-induced edema so it seems possible that chitosan blocks prostaglandins and cyclooxygenase release in later phase of acute inflammation.

Wound healing is an activephenomena including cellular, physiological and biochemical processes that result in restoration of connective tissue and formation of fibrous scar and lead to the repair of the anatomical continuity and functional status of the skin.[27] The procedure of wound-healing consists of four merged and overlapping stages: hemostasis, inflammation, proliferation, and tissue remodeling or resolution.[28]The current study was aimed to assess whether chitosan promote wound healing in experimentally induced wounds in rats. In excised wounds, since the edges are not in contact with each other contraction and epithelization are necessary for the repair process.

The results revealed that topical application of chitosan creams (5 and 10%) on excision wounds promoted contraction and period of epithelization of experimental wounds.Contraction of wounds is the process of driving healthful skin surrounding the wound to coat the naked area. The process of wound contraction is believed to be due to the action of myofibroblasts while epithelialization, which is the process of epithelial regeneration following damage, includes the proliferation and emigration of epithelial cells to the wound center.[29]Therefore, the influence of chitosan on the contraction and epithelialization of wounds suggest its possible enhancing effect on the migration and proliferation of epithelial cells, as well as the formation and action of myofibroblasts. Moreover, the increased capability of wound healing with chitosan could be explained on the basis of the anti-inflammatory effect that is well documented in the present study.

The earlier investigators reported the antimicrobial [10]and antioxidant[30]properties of chitosan. It was also reported that chitosan possessing hemostatic activity.[7] It is speculated that the antimicrobial and antioxidant properties could be one of the contributors for the wound healing effect of chitosan. Moreover, hemostatic effect of chitosan could also be responsible for wound healing activity.

CONCLUSION

In conclusion, chitosan has both an anti-inflammatory and wound healing activities. The probable mechanism of the wound healing activity of chitosan may be through its anti-inflammatory, antimicrobial and hemostatic effects. Further, isolation of active constituents from chitosan may bring about the development of a new wound-healing agent.

ACKNOWLEDGMENT

The authors are thankful to Deanship of Scientific Research (DSR), Prince Sattam bin Abdulaziz University, Al-Kharj, Saudi Arabia for providing the fund to carry out this study under research grants No. 5233/3/2015.

CONFLICT TO INTEREST: None

REFERENCES

1. Eshak M and Osman H. (2014). Biological Effects of Chitosan against Bisphenol- A Induced Endocrine Toxicity and Androgen Receptor Gene Expression Changes in Male Rats. IJPCR, 6(4): 300-311.
2. El-Denshary E, Aljawish A, El-Nekeety A, Hassan N, Saleh R, Rihn B and Abdel-Wahhab M. (2015). Possible Synergistic Effect and Antioxidant Properties of Chitosan Nanoparticles and Quercetin against Carbon Tetrachloride-Induce Hepatotoxicity in Rats. *Soft Nanoscience* Letters, 5: 36-51.
3. Yao HT, Luo MN, Hung LB, Chiang MT, Lin JH, Lii CK. (2012). Effects of chitosan oligosaccharides on drug-metabolizing enzymes in rat liver and kidneys. Food Chem Toxicol, 50(5): 1171-1177.
4. Santosh K, Joonseok K, Hyerim K, Gupta M and Dutta P. (2012). A New Chitosan Thymine Conjugates: Synthesis, Characterization and Biological Activity. International Journal of Biological Macromolecules, 50: 493-502.

5. Jeon Y and Kim S. (2001). Potential Immuno-Stimulating Effect of Antitumoral Fraction of Chitosan Oligosaccharides. Journal of Chitin Chitosan, 6: 163-167.
6. Vo T, Kim J, Ngo D, Kong C and Kim S. (2012). Protective Effect of Chitosan Oligosaccharides against Fc RI-Mediated RBL-2H3 Mast Cell Activation. Process Biochemistry, 47: 327-330.
7. Gua R, Sun W, Zhou H, Wu ZN, Meng Z, Zhu X. (2010). The Performance of a Fly-Larva Shell- Derived Chitosan Sponge as an Absorbable Surgical Hemostatic Agent. Biomaterials, 31: 1270-1277.
8. Ormrod D, Holmes C and Miller T. (1998). Dietary Chitosan Inhibits Hypercholesterolaemia and Atherogenesis in the Apolipoprotein E-Deficient Mouse Model of Atherosclerosis. Atherosclerosis, 138: 329-334.
9. Anraku M, Kabashima M, Namura H, Maruyama T, Otagiri M, Gebicki J and Tomida H. (2008). Antioxidant Protection of Human Serum Albumin by Chitosan. International Journal of Biological macromolecules, 43: 159-164.
10. Park P, Je J, Byun H, Moon S and Kim S. (2004). Antimicrobial Activity of Hetero-Chitosans and Their Oligosaccharides with Different Molecular Weights. Molecular Microbiology and Biotechnology, 14: 317-323.
11. Wenshui X, Ping L, Jiali Z and Jie C. (2011). Biological activities of chitosan and chitooligosaccharides. Food Hydrocolloids. 25: 170- 79.
12. Chae Y, Jang K and Nah W. (2005). Influence of molecular weight on oral absorption of water soluble chitosans. J Control Release. 102: 383- 94.
13. Abdou E, Nagy K and Elsabee M. (2008). Extraction and characterization of chitin and chitosan from local sources. Bioresource Technology, 99(5): 1359–1367.
14. Domard A and Rinaudo M. (1983). Preparation and characterization of fully deacetylated chitosan. International Journal Biological Macromolecules. 5: 49-52.
15. Ravindra R., Krovvidi K and Khan A. (1998). Solubility parameter of chitin and chitosan. Carbohydrate Polymers. 36: 121-127.
16. Tolaimate A, Desbrières J, Rhazi M, Alagui M, Vincendon M and Vottero P. (2000). The influence of deacetylation process on the physicochemical characteristics of chitosan from squid chitin. Polymer, 41: 2463-9.
17. Builders P, Kabele-Toge B, Builders M, Chindo B, Anwunobi P and Isimi Y. (2013). Wound Healing Potential of Formulated Extract from Hibiscus Sabdariffa Calyx. Indian J Pharm Sci, 75(1): 45–52.
18. OECD (Organization for Economic Cooperation and Development), 2001. The OECD 423 Guideline for Testing of Chemicals Acute Oral Toxicity-Acute Toxic Class Method, Paris, France.
19. Winter CA, Risley EA and Silber RH. (1968). Antiinflammatory activity of indomethacin and plasma corticosterone in rats. J Pharmacol Exp Ther. 162:196-201.
20. Mughrabi F, Hashim H, Ameen M, Khaledi H, Ali H, and Ismail S. (2011): Effect of Bis [benzyl N_-(indol- 3-ylmethylene)-hydrazinecarbodithioato]-zinc(II) derivatives on wound healing in Sprague Dawley rats. Indian Journal of Experimental Biology, 49(1): 50–55.
21. Muthu C, Ayyanar M, Raja N and Ignacimuthu S. (2006). Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu, India. ***Journal of Ethnobiology and Ethnomedicine***,2, article 43
22. Nayak B, Anderson M and Pereire P. (2007). Evaluation of wound-healing potential of catharanthus roseus leaf extract in rats. Fitoterpia, 78: 540-544.
23. Kean T, Roth S and Thanou M. (2005). Trimethylated chitosans as non-viral gene delivery vectors: Cytotoxicity and transfection efficiency. Journal of Controlled Release, 103(3): 643–653.
24. Buck W, Osweiter G and Van Gelder A. (1976). Clinical and Diagnostic Veterinary Toxicology. 2nd edn., Kendall/Hunt Publishing Co., Iowa, USA., ISBN-13: 9780840307200, p, 380.
25. Panthong A, Kanjanapothi D, Taesotikul T, Wongcome T and Reutrakul V. (2003). Anti-inflammatory and antipyretic properties of *Clerodendrum petasites* J. Ethanopharmacol, 85: 151-156.
26. Crunkhorn P and Meacock S. (1971). Mediators of the inflammation induced in the rat paw by carrageenan. Br J Pharmacol, 42, 392-402.
27. Bowler P. (2002). Wound pathophysiology, infection and therapeutic options. Annals of Medicine, 34(6): 419–427.
28. Gosain A and DiPietro L. (2004). Aging and Wound Healing. World Journal of Surgery, 28(3): 321–326.
29. Cotran R, Kumar V, Robbins S and Schoen F. (1994). Inflammation and Repair. In: Robbins Pathologic Basis of Disease, 5th Edn, W.B. Saunders Company, Pennsylvania, ISBN: 0-7216- 5032-5, pp: 51-92.
30. Kerch G. (2015). The Potential of Chitosan and Its Derivatives in Prevention and Treatment of Age-Related Diseases. Mar. Drugs, 13: 2158-2182.