



Experimental Study of Chitosan for Skin Wounds Healing in Rats

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Abstract

The present investigation's objective was to evaluate how Chitosan affected the healing of rats' full-thickness cutaneous wounds. All animals were generated with a clinically healthy (2 cm²) full-thickness dorsal back wound in 24 male adult rats (200- 250 g). Under the influence of a combination of 1 mg/kg of Acepromazine, 75 mg/kg of ketamine hydrochloride, and 10 mg/kg of xylazine hydrochloride. These animals were separated into three groups (A, B, and C) based on the treatment plan. The wounds in group (A) were treated locally with chitosan wound powder, whereas group (B) applied chitosan wound powder both locally and orally to treat the wounds, and group (C) left the wounds untreated as a control group. On 0, 3, 7, and 14 days after wound creation and treatment, four subgroups were formed for each group, having two wounds in each subgroup. Clinically, the result showed that wounds in group B healed faster than wounds in group A and group C. In addition, the results revealed that group (B) have enhanced cellularity, increased vasculature with the superiority of than those in both group (A) and group (C). Conclusion; The study show that the Chitosan work on increase wound healing and decrease Edam formation in the wound and replace the damaged part of the skin.

Keywords: wound healing; rat; chitin; chitosan.

Introduction:

Chitin, the primary component of many crustaceans' protective cuticles, including those found in crabs, shrimp, prawns, and lobsters, is transformed into chitosan via the process of alkaline deacetylation. (1). Chitosan's potential use in horticulture, agriculture, medicine, and food have been extensively studied (2).

Chitosan is the material of choice for biomedical applications since it has antimicrobial characteristics and is hydrophilic, biodegradable, biocompatible, and nontoxic. [3, 4]. Furthermore, it promotes granulation, vascularization, and migration of cells, and produces fibroblasts, which all contribute to wound healing [5,6,7]. Chitosan exhibits limited chemical strength and a fast rate of degradation despite its many benefits (8). Chitosan is offered commercially in a variety of forms with varying levels of deacetylation (50 percent to 95 percent) and molecular weight (about 300 to 1000 kDa) [9]. Chitosan becomes soluble in acidic solutions but is insoluble at physiological pH and in alkaline solutions when the amino group is protonated [10].

Following the chitosan formulation was administered orally or locally once daily, the study variables were established using rats as the animal model. The study's objective was to investigate the impacts of a treatment approach involving chitosan on the healing of cutaneous wounds and to evaluate the outcomes against a control group.

2. Materials and Methods

2.1 Experimental Animals

In the study, 24 adult male rats were separated into 3 groups with 8 rats, weighing between (200-250 gm). The animals were kept in separate cages in the University of Karbala's College of Veterinary Medicine animal house for the duration of the experiment. They were provided with a standard environment, which included food, care, and climate.

Ethical approve. The College of Medicine, University of Mosul, Iraq's Committee of Postgraduate Studies, officially approved the study protocol in accordance with the institutional regulations on animal handling and utilization research (UM.VET.2022.0).

2.2 Methods

2.3 Animals Preparation and Anesthesia

Food and liquids were denied for six to twelve hours before to the administration of anesthesia. Acepromazine was used as a pre-anesthetic to induce general anesthesia at a dose of 1 mg/kg body weight after the dorsal back area had been cleaned and shaved. The muscle was injected with 75 mg/kg bodyweight of ketamine and 10 mg/kg B.W of xylazine after 10 minutes (11). The rats were maintained in ventral recumbency while the dorsal aspect (back) of the animals was prepared to undergo aseptic surgery.

2.4 Surgical Operation

One square (2 cm²) full-thickness skin incisions were made on each animal's dorsal back. There were three groups of animals: group (A) had its rats' wounds treated with chitosan locally every day for seven days after the wound was first created; group (B) had its rats' wounds treated with chitosan both locally and orally every day; and group (C) had its rats' wounds left untreated.

2.5 Clinical Evaluations

All of the animals underwent a comprehensive clinical examination through 0,3,7 and 14 days during the period of the study. After each wound had been meticulously shaved, digital photographs were taken to see the wound's margin. Saline was used to carefully remove the scab from each wound, enabling improved tissue granulation and epithelization imaging.

2.6 Statistical Analysis

The System for Statistical Analysis was utilized [12]. The significance between the groups was determined using the Least Significant Difference –LSD test.

Results

3.1 Clinical Evaluation

The clinical interpretation of wounds extremely seemed to suggest that all treated wounds rapidly decreased in size during ongoing examination. In both treatment groups, day 3 marked the start of the change, and it became evident on day 7, particularly with regard to the injuries treated group (B). This pattern persisted until the examination was completed toward the end of the study, typically, distinct differences between the two therapy groups in terms of total damage recovery surfaced. (Figure 1). The accompanying wound healing process suggested that the rates of impact of fixation and daylong and the width of twisted withdrawal at third and seventh were not significantly different during the review's 14 days. On the fourteenth day of this review, the significant difference in progress between group (B) and the A and C groups

was evident (Table 1). At the same time, the effects of focus and day on the length and width of re-epithelization appeared in Table 2—which produced the same outcomes as in Table 1)

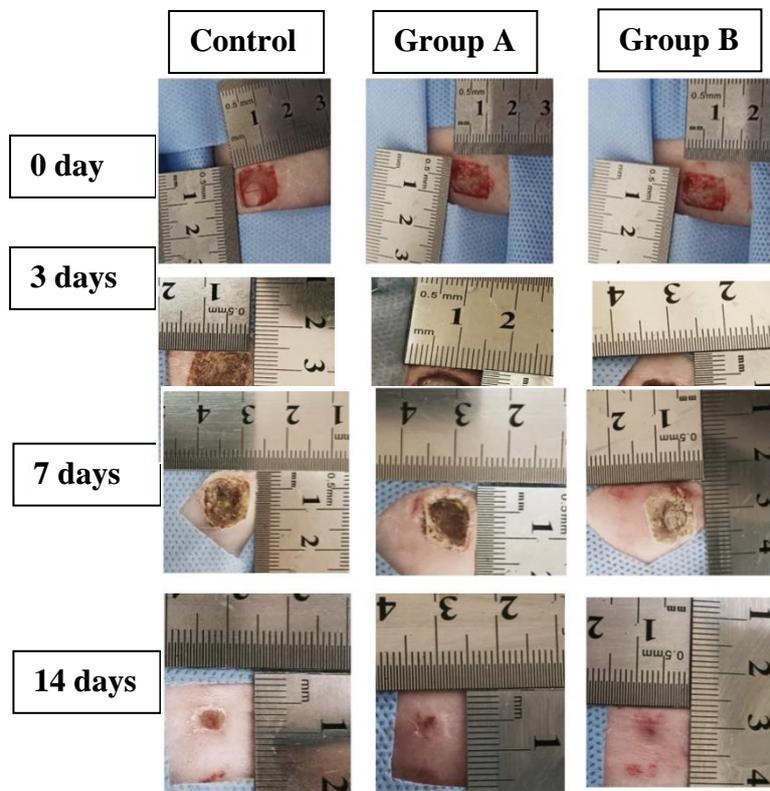


Figure 1: The total wound healing observed in the control (C), treated (A), and B groups was clearly different, at zero, 3rd, 7th, and 14th days post-treatment.

Table 1: Changes in the wound's length and width as a result of treatment and time

Days	Width			LSD value	length			LSD value
	A	B	C		A	B	C	
Day 0	1.9 ± 0.01	2 ± 0.01	2 ± 0.01	0.73 NS	2.00 ± 0.03	2.00 ± 0.00	2 ± 0.00	0.24NS
Day 3	1.9 ± 0.00	1.80 ± 0.00	2 ± 0.00	0.56 NS	1.8 ± 0.00	1.80 ± 0.00	1.8 ± 0.00	0.25 NS
Day 7	1.5 ± 0.00	1.40 ± 0.00	1.7 ± 0.00	0.83NS	1.60 ± 0.02	1.50 ± 0.00	1.70 ± 0.00	0.73 NS
Day 14	0.60 ± 0.00	0.30 ± 0.00	0.90 ± 0.00	0.1S	0.60* ± 0.00	0.40* ± 0.00	0.80 ± *	0.22

Table 2: The length and width of the wound's epithelization are affected by day and treatment methods. * (P≤0.05).

Days	Width			LSD value	length			LSD value
	A	B	C		A	B	C	
Day 0	1.9 ± 0.01	2 ± 0.01	2 ± 0.01	0.73 NS	2.00 ± 0.03	2.00 ± 0.00	2 ± 0.00	0.24NS
Day 3	1.9 ± 0.00	1.80 ± 0.00	2 ± 0.00	0.56 NS	1.8 ± 0.00	1.80 ± 0.00	1.8 ± 0.00	0.25NS
Day 7	1.5 ± 0.00	1.40 ± 0.00	1.7 ± 0.00	0.83NS	1.60 ± 0.02	1.50 ± 0.00	1.70 ± 0.00	0.73 NS
Day 14	0.60 ± 0.00	0.30 ± 0.00	0.90 ± 0.00	0.1S	0.60* ± 0.00	0.40* ± 0.00	0.80 ± 0*	0.22S

* (P≤0.05).

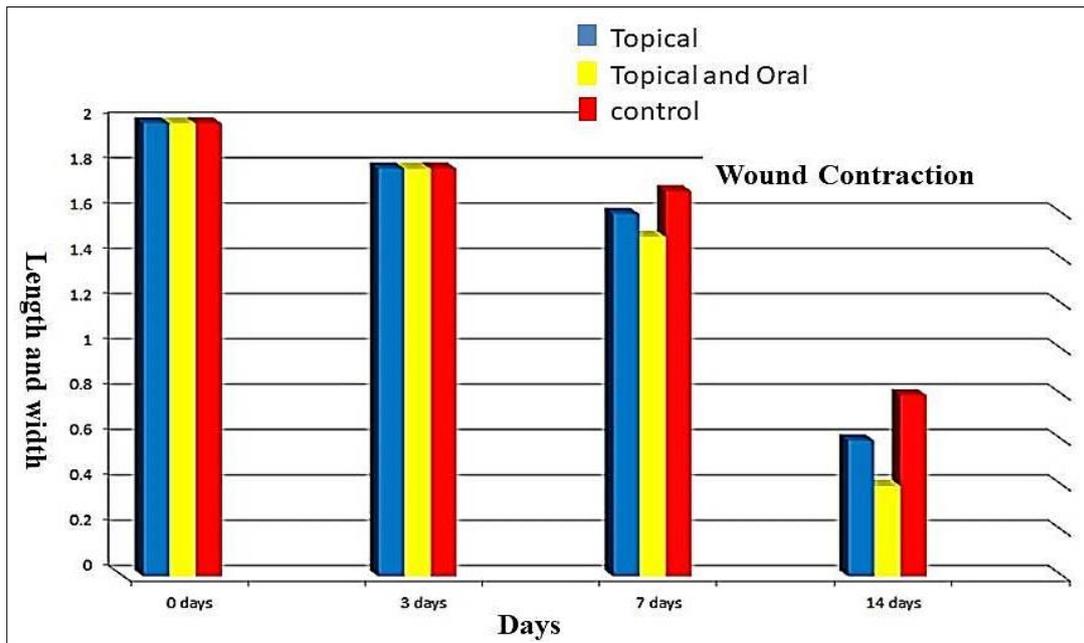


Figure 2: Contraction of the wound as a result of treatment methods and days

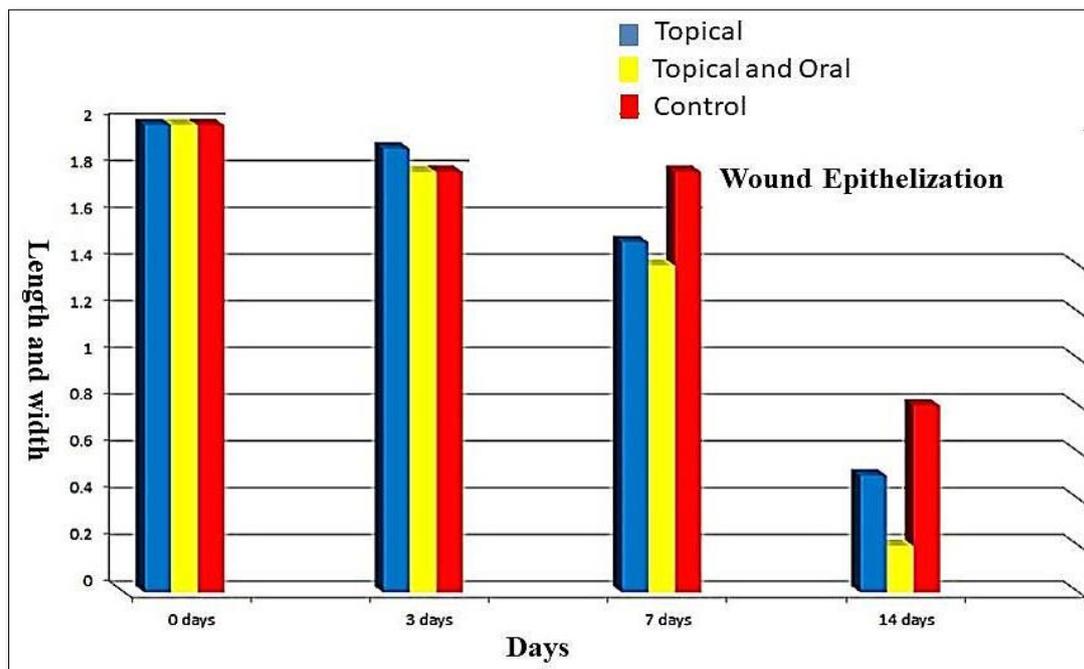


Figure 3: Effect of treatment methods and days on the wound epithelization.

Discussion

Clinical Evaluation

Chitosan and its derivatives are very biocompatible. They have antibacterial, antioxidant, anticancer, and immunomodulatory properties in addition to being biodegradable and harmless. Chitosan plays a vital part in the treatment of soft tissue illnesses, therefore it can be utilized to create various types of drug carriers depending on the intended usage (1).

The extensive usage of chitosan and its derivatives to replace or repair a portion of the tissue or the entire thing including the intervertebral disc, skin, cartilage, bone, blood vessels, nerves,

ligament, and tendon has been shown in numerous research (13, 14). The current study examines the healing effects of chitosan on full-thickness skin wounds in rats were evaluated and compared across all post-treated groups. According to the present study's clinical observations of the wounds, both treatment wounds' levels of healing progress started out quickly. However, the progression was greater in group B than it was in groups A and C, and it continued into the study's conclusion. Hydrogels made from chitosan and cellulose have been shown to speed up the formation of epithelial tissue on wounds, affect the structure of skin, stimulate skin restoration and contain a wealth of antimicrobial compounds to guard against wound infections. These findings are consistent with those of other animal studies (15). On the other hand, a study using chitosan hydrogels loaded with lithium chloride demonstrated a promising dressing for skin regeneration by considerably accelerating epithelial regeneration, promoting angiogenesis, and reducing wound inflammation (16). Furthermore, chitin and chitosan when use as biomedical applications in the treatment of cancer, wound healing, and drug delivery showed biocompatibility, non-immunogenicity, non-toxicity, and biodegradability (17). Also, this finding is in agreement with (18) in the study evaluation of chitosan's use as a wound dressing in rats.

Conclusions and Recommendations

Chitin can be converted into a biological material known as chitosan. Since it is a naturally occurring biodegradable, environmentally benign, non-toxic and biocompatible material, it is a highly interesting molecule. Chitosan is created by different degrees of chitin deacetylation. Both chemical and biological mechanisms can be used to create chitin. Conversely, chitosan is a material that has an enormous number of potential uses. Chitosan has applications in nearly every biological discipline. Chitosan is a potent antimicrobial agent that finds applications in food processing, farming, and healthcare. Chitosan has the potential to greatly influence regeneration technologies for the cornea, skin, and heart.

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