Herbal Medicines Journal **2021; 6(1):18-26**
DOI: 10.22087/hmj.v6i1.858 **ISSN:** 2538-2144

Original Article

A Comparison of Chitosan Gel and St. John's Wort Oil in Second-Degree Burns: An Experimental Study

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Received: 27.01.2021; Accepted: 25.11.2021

Abstract

Background and Aim: Hypericum perforatum L. (HP) and chitosan are materials that are abundant in nature with anti-inflammatory, analgesic, and antibiotic properties. The present study aimed to examine the effects of these two materials on burn injuries.

Materials and Methods: Twenty-one rats were divided into three groups, and burn injuries were experimentally created. Macroscopic healing of the wounds was evaluated by drawing the wound borders on transparent acetates once a week. At the end of four weeks, all the animals were sacrificed, and histopathological examinations were performed. The intact dorsum skin of the animals killed was excised to create the control group. A total of four groups were created, i.e. control, saline, St. John's Wort Oil (SJWO), and chitosan gel group, respectively. The results were compared statistically.

Results: At the end of the third week, the maximum reduction was observed in the SJWO group in the macroscopic examination ($p<0.05$). In histopathological examinations, dermal inflammation (Chi-square test, $p<0.05$) and epidermis thickness (Mann-Whitney U, $p<0.05$) were found to have the highest levels in the chitosan group.

Conclusion: All the preparations used in this study to treat burn injuries were effective, and the wounds improved compared to the initial state. SJWO was determined to provide the best healing of the burns. Long-term use of chitosan gel did not positively affect, with both macroscopic and histopathological findings showing that it delayed the healing of wounds compared to saline and SJWO.

Keywords: Hypericum, Chitosan, Burns

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Please cite this article as: Güler AG, Karakaya AE, Doğan AB, Bahar AY, Yazar FM. A Comparison of Chitosan Gel and St. John's Wort Oil in Second-Degree Burns: An Experimental Study. Herb. Med. J. 2021; 6(1):18-26.

Introduction

The treatment of burn injuries is a complex process that requires systemic and local treatments. The basic principle in the local treatment of burns is to protect the wound against bacteria, accelerate wound healing, and reduce the pain caused by the wound. Local wound care is performed using various local agents to help the wound heal quickly with less scarring. Combined treatments are needed to achieve all these.

In recent years, hypericum perforatum L. (HP) derivatives and gels containing chitosan have been frequently mentioned among the agents used for this purpose[1].

HP is a herb with antimicrobial, antidepressant, and anti-inflammatory properties[2]. It has a form called St. John's Wort Oil (SJWO), obtained by the maceration method by steeping it in olive oil. St. John's herb derivatives contain herbal combined active ingredients with anti-inflammatory, analgesic, and antibiotic properties (1, 2).

Chitosan is a hydrophilic, mucoadhesive cationic polyelectrolyte. It is prepared due to alkaline N-deacetylation of chitin, which is obtained from many creatures such as crabs, shrimps, and lobsters and has been proved to be effective in wound healing with antibiotics analgesic properties (3, 4). It is a natural polymer with swelling and gelling properties. A 1% glyceal acetic acid solution is often used to obtain the gel (5). To the best of our knowledge, there has been no previous comparative study in the literature of chitosan and St. John's Wort derivatives on wound healing.

Burn is a significant health problem that can occur in childhood, adulthood, and old age. The basic principle of treatment is to minimize burn damage. Studies are carried out with many substances associated with burns. Such studies are critical, and we consider it necessary to obtain more effective treatments (6). This experimental study aimed to examine and compare the healing effects of SJWO and chitosan on burn wounds.

Materials and Methods

Animals

All the procedures were performed according to the National Institutes of Health guide. Permission for this study was obtained from the Experimental Animals Local Ethics Committee (Ethical code: 09-2018/01) affiliated to the Faculty of Medicine, and the study was conducted in the Experimental Research Laboratory of Faculty of Medicine, Kahramanmaras Sutcu Imam University. A total of 21 Wistar albino rats, weighing 200-220 g, were kept in a room with ambient humidity between 45-65%, at a controlled temperature of 20-24°, with a 12-hour light-dark cycle. The animals had free access to water and rat food. Feeding was suspended 12 hours before the operation, but access to drinking water was continued. The rats were anesthetized with intramuscular ketamine (40 mg/kg) injection. The dorsum of each rat was shaved for localization of the burn. To create the burns, five conical steel plates, 2cm in diameter and weighing 42 g, were used in the study. The plates were kept in heat-controlled boiling water for at least 3 minutes and then applied to the shaved areas of the animals for 15 seconds without applying pressure (7, 8) (Figure.1). The 21 rats were then randomly separated into three groups of 7 and placed in cages labeled saline, SJWO, and chitosan gel groups.

Groups and Derivatives

Saline group: Serum saline was measured at 24 degrees pH 6.35 (pH meters, Sevencompact ph/ion meter s220, Mettler Toledo). This grouping aimed to give the rats the stress that occurs while administering the drugs to avoid the formation of differences between the groups.

SJWO group: Hypericum perforatum L. was dried and kept in a glass jar in 500cc of olive oil, placed under morning and evening sunlight of 50g of light for a total of 3 hours per day for 40 days until it turned red (9). SJWO was measured at 24 degrees ph 6.18 (pH meters, Sevencompact ph/ion meter s220, Mettler Toledo).

Chitosan gel group: Powdered chitosan (deacetylated ≥75%, from shrimp shells, sigma CAS: 9012-76-4) was mixed with 1% glyceal acetic acid until it became gel. It was kept in a covered container at room temperature for 4 hours to disappear air bubbles (5). Thus, the gel form was obtained. The pH of the gel form was measured at 24 degrees 5.80 (pH meters, Sevencompact ph/ion meter s220, Mettler Toledo).

Control group: It was created with tissue samples obtained from the intact skins of rats included in the groups mentioned above.

Treatment Protocol

The first treatment was administered to all rats 4 hours after the burn was applied. The treatment was applied as 0.5 ml every day, morning and evening for three weeks (7). Two people administered the treatment. While one person held the rat with a protective glove, the other person applied the preparation as 0.5 ml to the wound with a tube, then massaged the wound with

clean gloves for 5-10 seconds. No treatment was applied in the fourth week, at the end of which all the rats were sacrificed.

Macroscopic Study

After the rats were separated into three groups, the tail of each rat was numbered with an indelible pen. Each week, the wound border was drawn on transparent acetate with different colors. Attention was paid to have the same numbered rats in the groups during registration. ImageJ (ImageJ bundled with Java ver. 1.8.0_172) was then used to calculate the area within the wound boundaries. The marked area can be calculated by calibrating with the actual measurements on the photograph (Figure 2).

Histopathological Study

All the rats were sacrificed by cervical dislocation 28 days after the burns were created, and the wounds were excised from the intact margin. Equal size biopsies were taken from the symmetry of the wound in the dorsum of the sacrificed rats to form a control group. The biopsy material was fixed in 10% formaldehyde, then embedded in paraffin blocks. Sections were cut and stained with Hematoxylin-Eosin (HE), and all the samples were examined under a Nikon Eclipse Ni optical microscope. Crusting and bulla formation, dermal inflammation, neovascularization, fibroblast activity, collagen accumulation, and epidermis thickness were recorded using the histopathological scoring system (10). The scores were evaluated as yes (+) or no (-) for crusting, bulla formation and inflammation and as mild (+), moderate (++) or severe (+++) if polymorphonuclear leukocytes were <25%, 25%–50%, or>50%, respectively. Neovascularization, fibroblastic activity and collagen accumulation were recorded as none (-), mild (+), moderate (++) or severe (+++). Epidermis thickness was measured in micron units using NIS-Elements D 4.40.00 64-bit program.

Statistical Analysis

The data obtained in the study were analyzed statistically using IBM SPSS for Windows version 18.0 software (SPSS, Chicago, IL, USA). The

Figure 1. The stages of experimental burn formation in rats
A. Anesthesia to rats. B. Shaving the dorsum C. Creating burns with steel plates. D. Randomized placement in cages.

Repeated Measures ANOVA test was used for repeated measurements over time. When the Mauchly test was significant, Sera-Geisser adjustment was used to specify the statistical importance of determinants, ignoring the normality and variance distribution in the groups. The Kruskal – Wallis H test and Mann-Whitney U test were used to compare continuous variables without normal distribution. Chi-Square analysis was applied to categorical variables. Quantitative data were stated in the tables as median (minimum-maximum) values. A value of $p<0.05$ was accepted as statistically significant.

Results and Discussion

When the reduction in wound area average values over time was analyzed with the Repeated Measure ANOVA test, it was considered significant, and this significance indicated that healing occurred in all the groups, with differences seen between them (Figure 3, $p<0.05$). The weekly wound area measurements and the results of the statistical analysis have been presented in Table 1. No remarkable distinction was determined between the groups regarding the measured wound areas at the end of the first and second weeks. At the end of the third week, a noticeable distinction was found between the groups regarding the wound area measurements (Kruskal–Wallis H, Table 1). The Mann Whitney U-test results showed that the average wound area in the SJWO group was significantly smaller than that of the chitosan group ($p<0.05$).

At the end of 4 weeks, all the rats were sacrificed, and histopathological examinations were performed (Figure 4). Dermal inflammation, neovascularization, fibroblast activity, and collagen accumulation were evaluated, and the histopathological examination results were presented in Table 2. In comparing the groups of the parameters examined, dermal inflammation was significantly more severe in the chitosan gel group than in the other groups (Chi-square test, $p<0.05$).

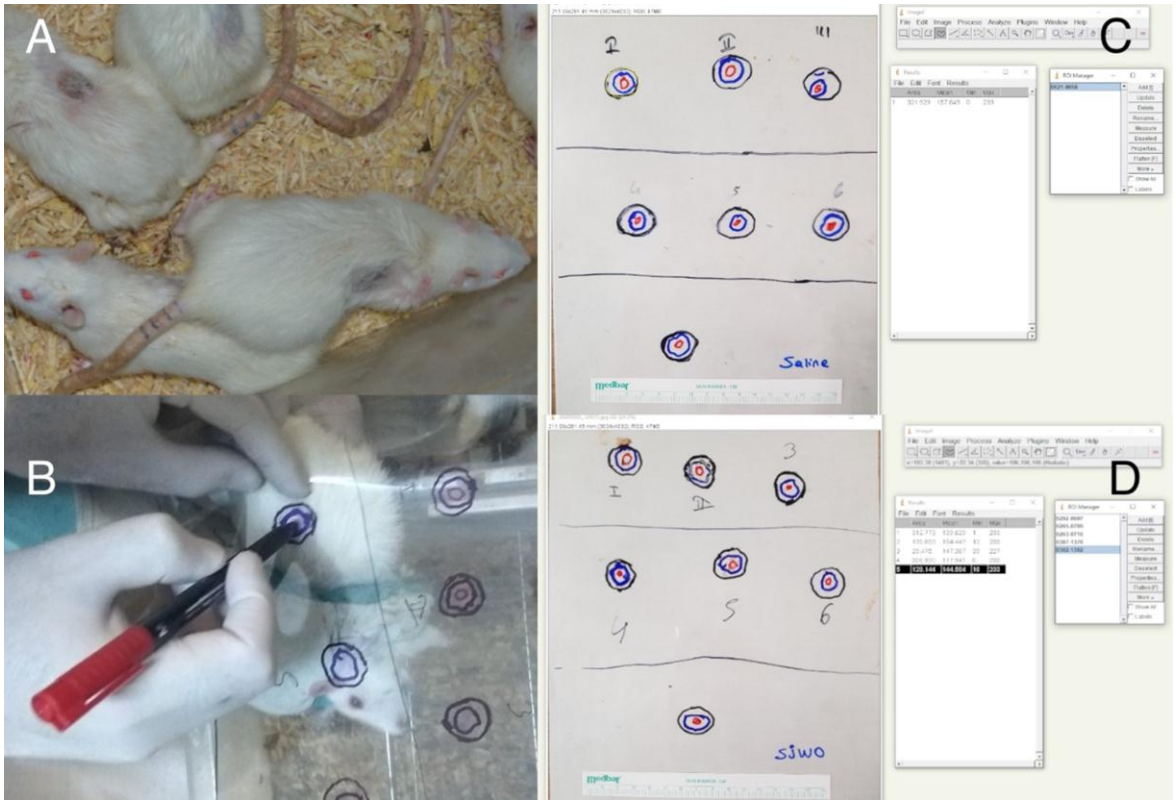

Figure 2. Tail markings of rats, drawing the wound border, measuring these drawings
A. Tail marks in rats. B. Drawing the wound boundaries. C. Measurement calibration and marking of weekly wound margins with ImageJ. D. Calculating the wound area with ImageJ and saving it to the computer.

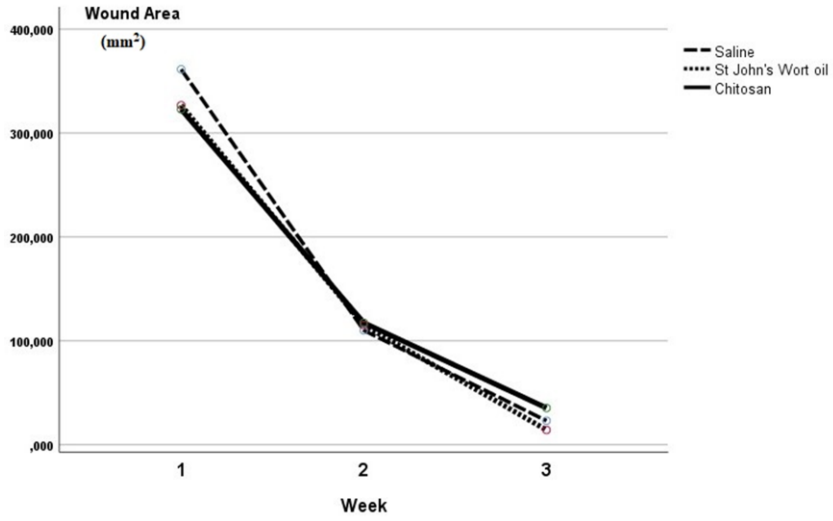

Figure 3. Graphs of reduction in wound area with weekly measurements.

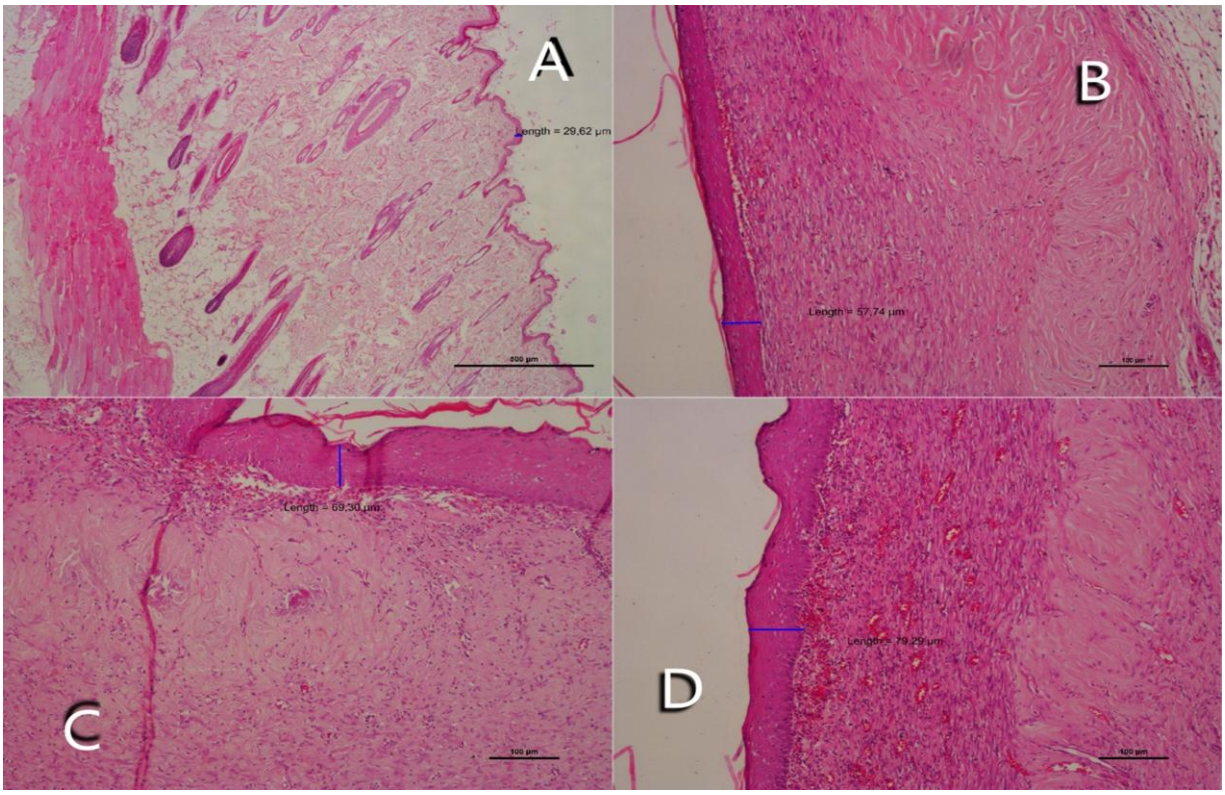

Figure 4. Histopathological examination

A. Control (x4 magnification, staining with H&E): Flabby connective tissue and numerous hair follicles observed under the thin epidermis layer. B. Saline (x10 magnification, staining with H&E): The epidermis has thickened, dermal structures and skin patches have been obliterated and replaced by inflammatory granulation tissue and fibrocollagenous tissue. C. SJWO (x10 magnification, staining with H&E): The epidermis has significantly thickened, there is significant accumulation of collagen in the reticular dermis, and inflammatory granulation tissue is present in the papillary dermis. D. Chitosan gel (x10 magnification, staining with H&E): Epidermis has significantly thickened, granulation tissue is cellular, there is dense inflammatory cell accumulation, and a low amount of fibrocollagenous tissue.

A more significant amount of collagen accumulation

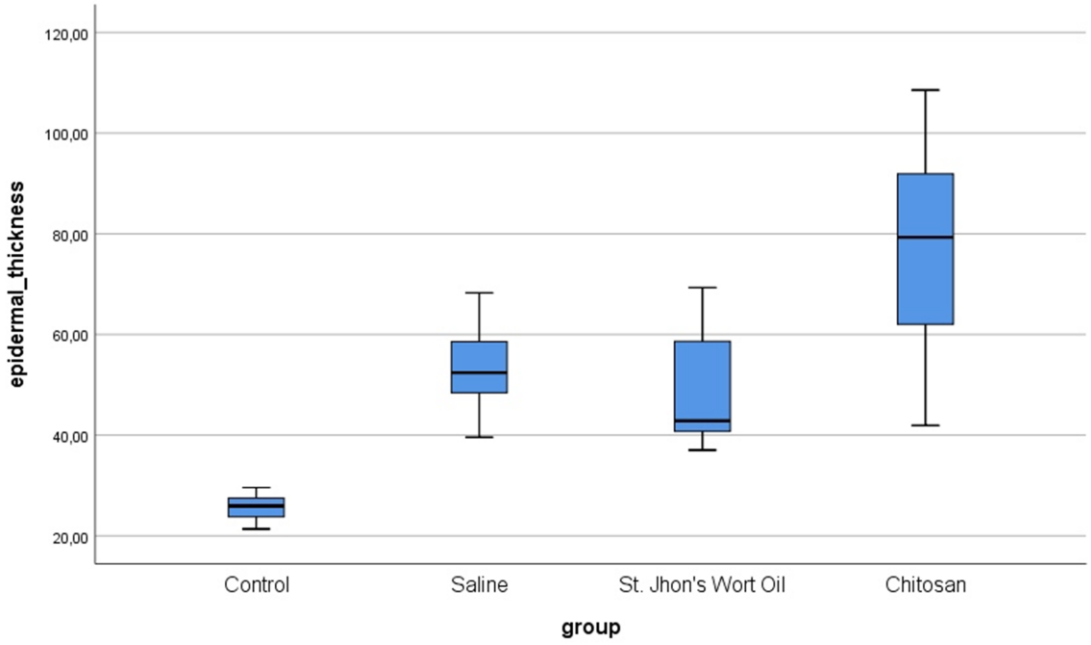

Figure 5. Comparison of epidermis thickness.

Table 1: Analysis of weekly measured wound area data in the macroscopic examinations.

Week	**Saline (mm^2) (n=7)**	**SJWO (mm^2) (n=7)**	**Chitosan (mm^2) (n=7)**	**p value**
1st week Median (Min.-Max.)	370.63 (312.75-384.36)	318.93 (302.31-364.02)	307.05 (251.50-393.84)	**0.152**
2nd week Median (Min.-Max.)	107.42 (79.20-157.81)	116.19 / (80.49-142.12)	119.68 / (92.46-150.81)	**0.561**
3rd week Median (Min.-Max.)	25.46 / (11.71-29.10)	14.08 / (5.14-26.92)	38.37 / (17.02-46.67)	**0.004**

Table 2: Histopathological examination results.

	Saline							**SJWO**							**Chitosan gel**							p
Number of rat	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7	
Crusting*	-	-	+	-	-	-	+	+	-	-	-	+	-	-	+	-	+	+	+	-	-	0.44
Dermal inflammation**	+	+	+	+	+	+	++	++	+	+	++	+	+	+	+++	+++	+++	+++	++	+	+	**0.03**
Neo-vascularization***	++	++	+	++	+	+	++	++	++	++	+	++	++	++	+	++	++	++	++	++	++	0.35
Fibroblast activity***	++	+++	++	+++	++	+	++	++	++	++	+++	++	++	++	++	++	++	++	++	+++	++	0.49
Collagen accumulation***	+	+	++	+	+	++	++	++	++	++	++	+++	++	++	+	++	+	+	++	++	++	0.08

* non-existent: -, ** mild: +, moderate: ++, severe: +++, *** mild: +, moderate: ++, severe: +++

was observed in the SJWO group but not at a significant level (p=0.08). The epidermis thickness in

Table 3: Examination results of epidermis thickness.

	Control	Saline	SJWO	Chitosan gel	p*
Median (Min.-Max.)	25.95 (21.37-29.60)	52.41 (39.57-68.27)	42.85 (37.02-69.30)	79.29 (41.93-108.56)	0.03

*Without control group

the control group was found to be significantly thinner than all the other groups ($p<0.05$). There was a significant difference between the groups regarding epidermis thickness (Table 3, Kruskal–Wallis H).

No statistically significant difference was found between the saline group and other groups in terms of epidermis thickness, while the epidermis thickness was found to be significantly thicker in the chitosan group than in the SJWO group (Figure.5, Mann-Whitney U, $p<0.05$). The treatment of human burn injuries is lengthy, costly and troublesome (11, 12). Several experimental studies have been conducted to obtain new effective and affordable preparations (13, 14), and have revealed that materials combined with chitosan gel and prepared in the form of pledgets are quite effective (15, 16). One of those studies reported the ideal period for pledget uses to be three days and that maintaining the bandage for nine days significantly delayed wound healing (16). Several studies in the literature have shown that chitosan accelerates wound healing in the early period. In one of those studies, chitosan gel combined with epidermal growth factor (EGF) was more effective than the application of chitosan only (17). In the same study, the results of biopsies taken on days 3, 7, and 14 after wound formation revealed that epidermis thickness was highest in the group applied with chitosan combined with EGF, which was interpreted as the combination of chitosan with EGF having the ability to accelerate the healing of burn wounds.

However, in a study conducted by Ueno *et al.*, a 2x2 cm wound was created in the dorsum of dogs, and three-day wound dressings were applied with chitosan with 18% deacetylation. Initially, wound healing was more rapid on the side where chitosan was administered, but type III collagen secretion accelerated in the late period, which resulted in granulation increase (18). The granulation tissue thickness was found to be higher than that of the control group on the 15th day after wound formation. Therefore, it was emphasized that attention should be paid to the duration of treatment with chitosan, as excessive granulation can cause a delay in epithelialization, thereby delaying wound healing. Although studies have shown that treatment with chitosan is effective in the early period, there has always been a lack of clarity about late periods. Macroscopic findings of wound healing have not been specified in those studies since wound biopsies were performed in the early period. The current study differs in that the wound healing was observed since the experimental animals were not biopsied during the treatment period. Another difference is that the wound area was measured, and there was a significant slowing down in the wound healing after the 2nd week in the group where chitosan was administered. Histopathological examinations supported these findings and wound healing was found to be the slowest in the chitosan group. Considering that histopathological examination was performed after four weeks, wound healing was delayed in the chitosan group. Okamoto *et al.* studied the effects on dogs using the powder form of chitosan with at least 80% chitin and deacetylation and reported that inflammatory cells decreased significantly in the chitosan group but significantly increased in the control group after 28 days (19). However, the present study revealed contrasting findings, as dermal inflammation was determined to be significantly higher in the chitosan gel group at the end of 28 days. This difference might be due to the powder form of chitosan in the Okamoto study, as the gel form of chitosan gel creates a thin film layer on the wound

after it dries. This prevents the wound from receiving the gases it needs, such as oxygen. It has been demonstrated many times that opening wound dressings early or dressing them in a way that does not prevent air from circulating accelerates healing. However, in an outstanding experimental study by Sano *et al*., symmetrical wounds were created in the dorsum of 6 rats, and oxygen impermeable (polyvinylidene chloride) as well as permeable (polymethylpentene) membranes were placed on the wounds. The study results showed that wound healing was delayed in the animals applied with impermeable membranes (20). As chitosan forms a semipermeable film, its use in the food industry has been studied (21). There is much information in the literature that it can be used as an edible food preservative film as it develops a layer that protects food from contact with air (22, 23). This explains the similarity of the current study results with those of Sano *et al*.

The use of HP on burn injuries is also mentioned in the famous medical book, Canon of Medicine (al-Qanun-fi-al-tibb), by Ibn Sina (980-1037 AD). SJWO is rich in hypericin as it is obtained from the HP plant, and it is widely known that hypericin is effective in wound healing (24). In an experimental study by Sayar *et al*., second-degree burns were created in 21 rats separated into three groups and administered phenytoin, hypericin, and saline, respectively. It was concluded that the healing in the hypericin group was more remarkable than in the groups administered phenytoin and saline. Studies conducted on the use of SJWO have shown it effective in treating corrosive esophageal burns and protecting against myringosclerosis in skin burns (7, 5, 26). As a result of both the macroscopic and microscopic findings of the present study, SJWO was determined to be effective in treating burn injuries. Although no statistically significant difference was found between SJWO and saline, the most effective material used in the experiment was determined to be SJWO.

Conclusion

There is no study in the literature comparing SJWO and chitosan gel on the healing of burn wounds. This experimental study showed that long-term use of chitosan gel alone prolonged burns wound healing duration compared to SJWO and saline.

Funding

This work was supported by a grant from the Kahramanmaraş Sütçü İmam University Scientific Research Projects Unit, Project Number: 2015/4-19 M

Acknowledgment

None.

Conflict of Interest

The authors declare that they have no conflict of interest.

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