EXPERIMENTAL STUDY

Histopathological investigation of the effect of chitosan on oral mucous wound healing in experimentally established diabetic rats

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ABSTRACT

BACKGROUND: In our study, it was aimed to histopathologically investigate the effects of chitosan on wound healing in the oral mucosa by applying the gel form of experimentally induced diabetes mellitus.

METHODS: In our study, 42 male Sprague Dawley rats weighing 340±20 g, 14–16 weeks old, were used. Diabetes induction was achieved by administering 55 mg/kg streptozotocin intraperitoneally (i.p.) to 32 of the subjects. Those with blood glucose levels above 250 mg/dl as measured at the end of the 2nd and 7th days were considered diabetic and included in the study. Afterwards, a wound of 5 mm in diameter and 1 mm in depth was created in the buccal mucosa of the experimental animals with a disposable punch biopsy tool. Wound healing was evaluated on the 2nd and 5th days after the surgical operation. The samples were evaluated histopathologically in terms of inflammation, fibrosis, epithelial regeneration, necrosis, and foreign body reaction.

RESULTS: As a result of the statistical analysis, a significant difference was found between the groups in terms of inflammation levels on the 2nd and 5th days ($p<0.05$). In the intragroup evaluations, the rate of severe inflammation on the 2nd day in the diabetes+chitosan group was found to be statistically significantly higher than the 5th day ($p<0.05$). While there was no statistically significant difference between the groups in terms of fibrosis levels on the 2nd day ($p>0.05$), a statistically significant difference was found in terms of fibrosis levels on the 5th day ($p<0.05$).

CONCLUSION: It was observed that chitosan did not cause foreign body reaction in any of the groups on the 2nd and 5th days.

Keywords: Chitosan; histopathology; wound.

INTRODUCTION

Diabetes mellitus (DM) is an endocrine system disease characterized by hyperglycemia along with the defect in carbohydrate, lipid, and protein metabolism that occurs due to total or partial deficiency of insulin produced by β cells in the islets of Langerhans in the pancreas.

Hyperglycemia occurring in individuals with diabetes; It has a negative effect on the healing process by reducing the formation of fibroblast, which is one of the factors that play a role in wound healing, inhibiting cell proliferation and collagen tissue formation, reducing the formation of granulation tissue, and increasing the risk of infection.[1]

In addition to the current methods applied in the wound treatment of diabetic patients, it is of great importance to carry out new studies to contribute to the treatment process. In recent years, studies on biopolymers have been carried out to investigate the effects of diabetic patients on wound healing.[2]

Chitin is the second most abundant biopolymer in nature after cellulose. Although there are many derivatives of chitin,

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the most important among them is chitosan. Studies have shown that chitosan accelerates wound healing and bone formation, prevents adhesion in the post-operative period, and also has antibacterial and hemostatic effects.[3] In addition, it has been stated that chitosan is effective in all stages of wound healing in experimental animal models. It has been shown that chitosan has hemostatic activity in the inflammation phase, and it has been stated that it regulates the migration of macrophages and neutrophils, and also affects the formation of granulation tissue.[4]

In our study, we aimed to examine the effectiveness of chitosan, which we know to have a positive effect on wound healing, on the oral mucosa, unlike other studies on the skin, by experimentally creating wounds on the oral mucosa of rats with whom we had diabetes.

MATERIALS AND METHODS

In our study, 42 male Sprague Dawley strains of 14–16 weeks old rats weighing 340±20 g were used. Experimental animals were kept at 22±1°C throughout the experiment, in a room with 12 h of light and 12 h of dark, in a metal cage with 1 experimental animal. The subjects were fed with standard rat chow and tap water. Ethics committee approval of this study was given by Istanbul University Animal Experiments Local Ethics Committee on May 25, 2018.

Experimental Induction of Diabetes by Streptozotocin (STZ)

To induce diabetes, STZ was administered to the experimental animals after 12 h of fasting. STZ (Sigma S0130-100 MG), which is used to experimentally induce diabetes, was administered intraperitoneally (i.p) at 55 mg/kg to each animal to be diagnosed with diabetes.

Chitosan Gel Preparation

One gram of chitosan was added to 50 ml of 1% acetic acid solution. The prepared mixture was stirred for about 1 h until the chitosan particles were completely dissolved, and a chitosan solution was obtained. The pH of the solution was adjusted to 4. The solution was left under UV light for 1 night to remove air bubbles and sterilize it. After the solution took the gel form, it was taken into glass jars with the mouth closed and made suitable for use.

Surgical Method

The weight of the experimental animals to be used in the study was measured before the operation and the amount of anesthesia to be given was determined. General anesthesia was achieved by intraperitoneal administration of a mixture of 50 mg/kg Ketamine HCl and 10 mg/kg Xylazine HCl. With a 1-time punch biopsy, a wound of 5 mm in diameter and 1 mm in depth was created in the buccal mucosa of the animals, and then left for secondary healing.

Formation of Experimental Groups

Forty-two Sprague Dawley male rats used in the study were divided into three main groups and a total of 6 subgroups arranged according to the scarifications performed on the 2nd and 5th days following the surgical operation.

Group 1 (Control Group [K]): 10 experimental animals were used in this group. Two subgroups were formed, with 5 animals sacrificing on the 2nd day after the surgical operation and 5 animals with scarification on the 5th day after the surgical operation.

The animals in this group were injected with saline instead of STZ to create the same stress as the experimental animals in the Diabetes (D) and Diabetes+Chitosan (DK) groups. After the 12-h fasting period of the animals, 0.05 ml of blood was taken from the tail veins and the measurement was performed with a glucometer device. After measuring the blood sugars of the rats, it was observed that it was below 250 mg/dl. Until the day of the surgical operation, sugar and weight follow-ups were made regularly. On the day of the operation, the punch biopsy tool was used once, and a wound was created on the buccal mucosa and the wound was left to heal secondary. Fasting blood glucose (FGL) of 5 rats to be sacrificed on the 2nd day was first measured with a glucometer, and then scarification was performed. Five rats in the other subgroup were sacrificed after measuring their FGL on the 5th day. The buccal mucosa of the rats was excised excisionally and placed in 10% formol solution.

Group 2 (Diabetes Group [D]): A total of 16 experimental animals were used so that 8 experimental animals were sacrificed 2 days after the surgical operation, and eight experimental animals were sacrificed 5 days after the surgical operation.

After the experimental animals were fasted for 12 h, 0.05 ml blood sample was taken from the tail veins and it was determined with a glucometer device that they were in normal blood sugar before diabetes. Afterward, 55 mg/kg STZ dose was adjusted with precision balance in microfuge tubes. After STZ was dissolved homogeneously in a freshly prepared citrate buffer solution each time, it was administered intraperitoneally to the rats. Afterward, 5% glucose solution was added to drinking water for the first 24 h to prevent sudden hypoglycemia expected to occur in rats. Two days after STZ was injected, blood samples were taken from the tail veins in the same way, and blood glucose was measured. It was determined that the blood sugar of the rats was higher than 250 mg/dl and diabetes induction was successful. Then, the rats were kept under observation for 5 days for the development of diabetes effects. The weight of the animals was monitored daily. One week after diabetes induction, the surgical operation phase was started and a wound was created on the buccal mucosa. Eight an-

imals in the first subgroup were sacrificed 2 days after the operation, after measuring their FGL with a glucometer. After the buccal mucosa was excised excisionally, they were placed in 10% formol solution. On the 5th day after the operation, FGL of 8 animals in the second subgroup was measured and the animals were sacrificed. The buccal mucosa of the rats was also removed and placed in 10% formol solution.

Group 3 (Diabetes+Chitosan Group [DK]): A total of 16 experimental animals consisting of two subgroups were used in this group. As in the other main groups, the scarification times were on the 2nd and 5th days after the surgical operation, and 8 experimental animals were used in both subgroups.

The blood glucose measurements of the experimental animals were performed with 0.05 ml blood sample taken from the tail veins. It was determined that blood sugar levels of all animals were below 250 mg/dl. Freshly prepared streptozocin was injected into the rats by dissolving it in citrate buffer at a dose of 55 mg/kg each time. A 5% glucose solution was added to drinking water to prevent sudden hypoglycemic shock, which was predicted to occur in the first 24 h. Two days after the injection, the blood glucose levels of the rats were determined with samples taken from the tail veins. It was determined that the blood sugars of the experimental animals were above 250 mg/dl. Afterward, IT was followed for 5 days for the development of diabetic effects. Weight monitoring was continued every day. At the end of the 1-week period, blood samples were taken from the tail veins and blood glucose was measured using a glucometer device. Following the process, the surgical operation stage was started. A wound was created on the buccal mucosa with a disposable punch biopsy tool. Chitosan gel, prepared in appropriate consistency, was applied to the relevant wound area after bleeding control. Two days after the operation, the first subgroup was sacrificed by measuring FGL with a glucometer device. The buccal mucosa of the rats was removed and placed in 10% formol solution. Rats in the second subgroup were sacrificed after measuring their FGL on the 5th day. The excised buccal mucosa samples were placed in 10% formol solution.

Histopathological Reviews

After scarification, the cheeks of the experimental animals were excised excisionally and fixed in 10% buffered formalin solution for 1 week. After fixation, sections passing through the largest diameter of the ulcer area were taken and routine tissue follow-up was performed, and paraffin blocks were prepared. Sections of 3 micron thickness obtained from these blocks were stained with hematoxylin-eosin and examined under a light microscope. % calculations were made on the digital photographs taken using the "Olympus analysis 5" (Tokyo-Japan) image analysis system. The histopathological criteria examined are given in Table 1.

Statistical Analysis

While evaluating the findings obtained in the study, IBM SPSS Statistics 22 (IBM SPSS, Turkey) program was used for statistical analysis. While evaluating the study data, the conformity of the parameters to the normal distribution was evaluated with the Shapiro–Wilk's test. While evaluating the study data, the One-way ANOVA test was used for the comparison of the normally distributed parameters in the comparison of the quantitative data, and the Tukey HDS test and Tamhane's T2 test were used to determine the group that caused the difference. Student's t-test was used for the comparison of normally distributed parameters between two groups. Paired Sample t-test was used for within-group comparisons of normally distributed quantitative data. Chi-square test, Fisher's Exact test, and Fisher Freeman Halton test were used to compare qualitative data. Significance was evaluated at $p<0.05$ level.

RESULTS

Body Weight Findings

There was no statistically significant difference between the groups in terms of weight averages on day 0, day 2, and day 7 ($p>0.05$).

There was a statistically significant difference between the groups in terms of 9th day weight averages ($p=0.027$; $p<0.05$). As a result of the pairwise comparisons made to determine which group the difference originates from; the mean 9th day

Table 1. Histopathological criteria results

	Histological	Evaluation	Criteria	
	0	1	2	3
Inflammation	None	Mild	Middle	Severe
Fibrosis	None	1%–20	20%–60	More than 60%
Epithelialization	None	1%–20	20%–60	More than 60%
Necrosis	None	Have	–	–
Foreign body reaction	None	Have	–	–

weight of the control group was found to be significantly higher than the Diabetes Group and Diabetes+Chitosan Groups (p1=0.040; p2=0.039; p<0.05). There was no statistically significant difference between the Diabetes Group and Diabetes+Chitosan groups in terms of 9th day weight averages (p>0.05).

In the control group; the increases observed in the average weight of the 7th and 9th days compared to the initial weight average were statistically significant (p<0.05).

In the diabetes group; the decreases observed in the weight averages of the 2nd, 7th, and 9th days compared to the initial weight average were statistically significant (p<0.05).

In the Diabetes+Chitosan group; the decreases observed in the weight averages of the 2nd, 7th, and 9th days compared to the initial weight average were statistically significant (p<0.05).

Fasting Blood Sugar Findings

There was no statistically significant difference between the groups in terms of the mean of FGL on day 0 and day 2 (p>0.05).

There was a statistically significant difference between the groups in terms of FGL averages on the 7th day (p=0.000; p<0.05). As a result of the pairwise comparisons made to determine which group the difference originates from; the mean 7th day FGL of the control group was found to be significantly lower than the Diabetes Group and Diabetes+Chitosan Groups (p1=0.000; p2=0.000; p<0.05). There was no statistically significant difference between the Diabetes Group and Diabetes+Chitosan groups in terms of 7th day FGL averages (p>0.05).

There was a statistically significant difference between the groups in terms of 9th day FGL averages (p=0.000; p<0.05). As a result of the pairwise comparisons made to determine which group the difference originates from; the mean 9th day FGL of the control group was found to be significantly lower than the Diabetes Group and Diabetes+Chitosan Groups (p1=0.000; p2=0.000; p<0.05). There was no statistically significant difference between the Diabetes Group and Diabetes+Chitosan groups in terms of 9th day FGL averages (p>0.05).

In the control group; there was no statistically significant change in the mean FPG on the 7th, 9th, and 12th days compared to the initial mean FPG (p>0.05).

In the diabetes group; the increases in the mean FGL on the 2nd day, 7th day, 9th day, and 12th day compared to the initial FGW mean were statistically significant (p<0.05).

In the Diabetes+Chitosan group; the increases in the mean FGL on the 2nd day, 7th day, 9th day, and 12th day compared to the initial FGW mean were statistically significant (p<0.05).

Histopathological Findings

There was a statistically significant difference between the groups in terms of inflammation severity levels on the 2nd day (p<0.05). As a result of the pairwise comparisons made to determine which group the difference originates from; the difference was found between the control and diabetes groups (p=0.001; p<0.05). There was no statistically significant difference between the other groups in terms of the 2nd day inflammation severity levels (p>0.05).

There was a statistically significant difference between the groups in terms of inflammation severity levels on the 5th day (p<0.05). As a result of the pairwise comparisons made to

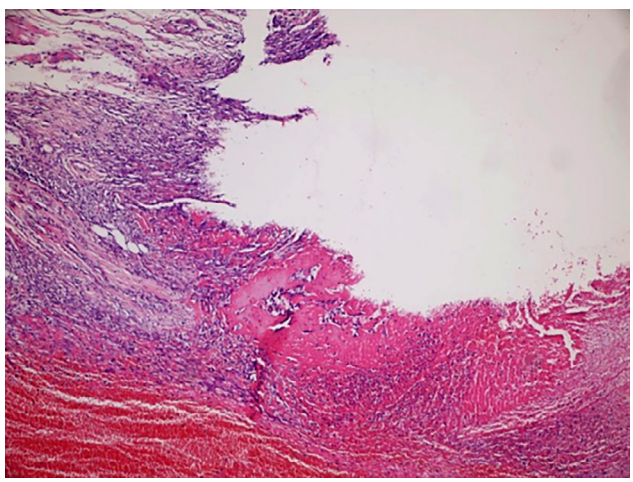

Figure 1. On the 2nd day, the stratified squamous epithelium covering the surface disappeared in large areas in the control group. Exudate and debris cover these areas. Beneath these are areas of hemorrhage and fiber-rich connective tissue with infiltration of neutrophil polymorphs, lymphocytes, and few plasma cells (H&E ×100).

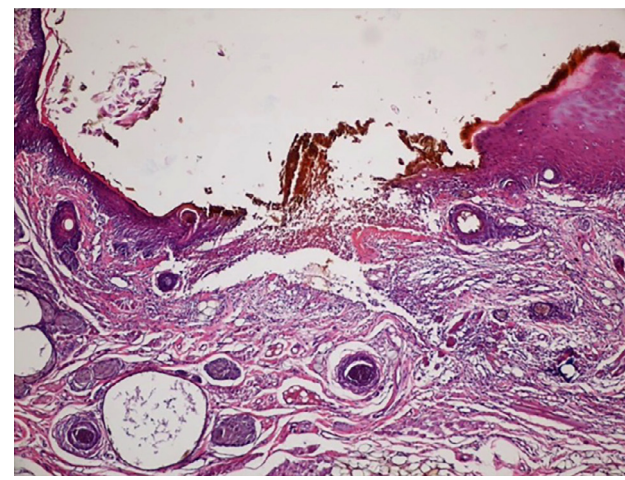

Figure 2. On the 5th day, in the control group, it is observed that the surface epithelium regenerates from the wound edges and narrows the ulcer area. There is less inflammatory cell infiltration than the 2nd day group (H&E ×100).

determine which group the difference originates from; a difference was found between the control group and the Diabetes and Diabetes+Chitosan groups (p=0.001; p<0.05). A difference was found between the diabetes group and the Diabetes+Chitosan group (p=0.001; p<0.05).

There was no statistically significant difference between the groups in terms of 2nd day necrosis rates (p>0.05). On the 2nd day, 80% of the control group, 100% of the diabetes group, and 100% of the Diabetes+Chitosan group had necrosis.

There was no statistically significant difference between the groups in terms of the incidence of necrosis on the 5th day (p>0.05). On the 5th day, 80% of the control group, 87.5% of the diabetes group, and 100% of the Diabetes+Chitosan group had necrosis.

There was no statistically significant difference between the groups in terms of 2nd day fibrosis levels (p>0.05).

There was a statistically significant difference between the groups in terms of 5th day fibrosis levels (p<0.05). As a result of the pairwise comparisons made to determine which group the difference originates from; the difference was determined between the Control and Diabetes+Chitosan groups (p=0.007; p<0.05). There was no statistically significant difference between the other groups in terms of fibrosis levels on the 5th day (p>0.05).

There was a statistically significant difference between the groups in terms of epithelial regeneration levels on day 2 (p<0.05). As a result of the pairwise comparisons made to determine which group the difference originates from; a difference was found between the control group and the diabetes and Diabetes+Chitosan groups (p=0.007; p<0.05). There was no statistically significant difference between the diabetes and Diabetes+Chitosan groups in terms of epithelial regeneration levels on the 2nd day (p>0.05).

There was a statistically significant difference between the groups in terms of epithelial regeneration levels on the 5th day (p<0.05). As a result of the pairwise comparisons made to determine which group the difference originates from; a difference was found between the control group and the diabetes and Diabetes+Chitosan groups (p1=0.032; p2=0.007; p<0.05). There was no statistically significant difference between the diabetes and Diabetes+Chitosan groups in terms of epithelial regeneration levels on the 5th day (p>0.05). There was a statistically significant difference between the groups in terms of 5th day fibrosis levels (p<0.05). As a result of the pairwise comparisons made to determine which group the difference originates from; the difference was determined between the control and Diabetes+Chitosan groups (p=0.007; p<0.05). There was no statistically significant difference between the other groups in terms of fibrosis levels on the 5th day (p>0.05).

There was a statistically significant difference between the groups in terms of epithelial regeneration levels on day 2 (p<0.05). As a result of the pairwise comparisons made to determine which group the difference originates from; a difference was found between the control group and the diabetes and Diabetes+Chitosan groups (p=0.007; p<0.05). There was no statistically significant difference between the diabetes and Diabetes+Chitosan groups in terms of epithelial regeneration levels on the 2nd day (p>0.05).

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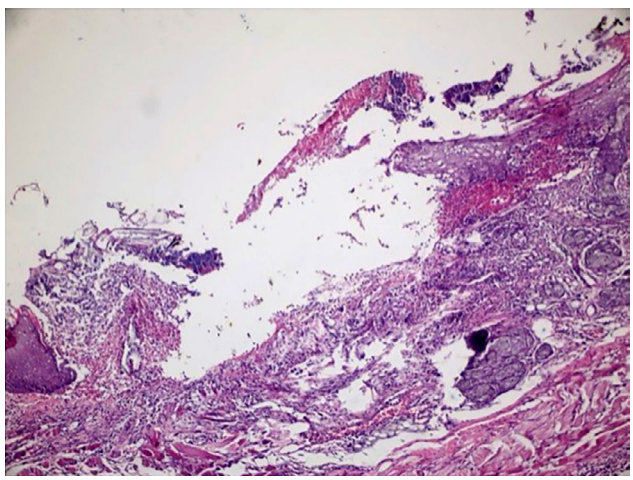

Figure 3. On the 2nd day, the surface epithelium cannot be observed in a large area in the diabetes group. These areas are covered with exudate, debris, and fibrin aggregates. Intense inflammatory cell infiltration is detected in the underlying connective tissue (H&E ×100).

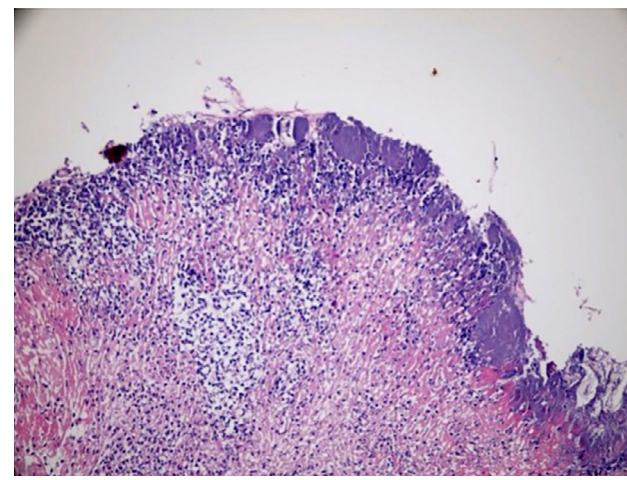

Figure 4. On the 5th day, the surface ulcer area in the diabetes group showed minimal narrowing compared to the 2nd day diabetes group, but it was again found to be covered with exudate and debris as a large area. There is deep connective tissue with intense inflammatory cell infiltration and bleeding areas (H&E ×100).

difference was found between the control group and the diabetes and Diabetes+Chitosan groups (p1=0.032; p2=0.007; p<0.05). There was no statistically significant difference between the diabetes and Diabetes+Chitosan groups in terms of epithelial regeneration levels on the 5th day (p>0.05).

In the control group;

- The rate of moderate inflammation on the 2nd day was found to be statistically significantly higher than on the 5th day (p<0.05).
- There was no statistically significant difference in the incidence of necrosis between the 2nd and the 5th day (p>0.05).
- The rate of fibrosis being 1–20% of the ulcer area on the 2nd day was found to be statistically significantly higher than on the 5th day (p<0.05).

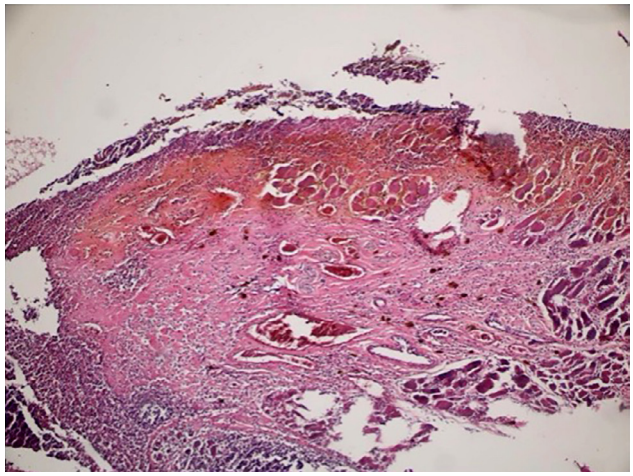

Figure 5. On the 2nd day in the diabetes + chitosan group, the stratified squamous epithelium covering the surface disappeared in large areas, and exudate, debris and microorganism colonies were covering these areas. Beneath these is loose connective tissue with dense inflammatory cell infiltration (H&E ×200).

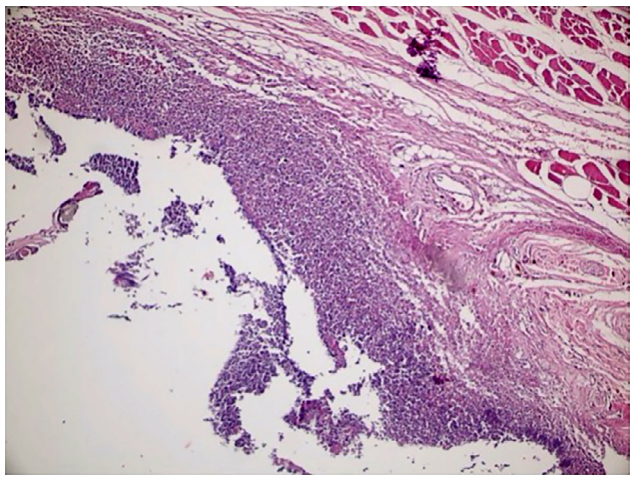

Figure 6. In the 5th day diabetes + chitosan group, the width of the ulcer area appears to be minimally smaller than the 2 day diabetes + chitosan group. Underlying there is extensive inflammatory cell infiltration (H&E ×100).

- The rate of epithelial regeneration being 1–20% of the ulcer area on the 2nd day was found to be statistically significantly higher than on the 5th day (p<0.05).

In the diabetes group;

- There was no statistically significant difference between the 2nd day and the 5th day in terms of the severity of inflammation (p>0.05).
- There was no statistically significant difference in the incidence of necrosis between the 2nd and the 5th day (p>0.05).
- The rates of fibrosis in 1–20% of the ulcer area and 20–60% of the ulcer area on the 2nd day were found to be statistically significantly lower than on the 5th day (p<0.05).
- The rates of epithelial regeneration in 1–20% of the ulcer area and 20–60% of the ulcer area on the 2nd day were found to be statistically significantly lower than on the 5th day (p<0.05).

In the Diabetes+Chitosan group;

- The rate of severe inflammation on the 2nd day was found to be statistically significantly higher than on the 5th day (p<0.05).
- The rate of fibrosis of 1–20% of the ulcer area on the 2nd day was found to be statistically significantly lower than on the 5th day (p<0.05).
- The rate of epithelial regeneration being 1–20% of the ulcer area on the 2nd day was found to be statistically significantly lower than on the 5th day (p<0.05).

DISCUSSION

Today, many biomaterials are used to help heal diabetic wounds. One of them is chitosan and it has been stated that it has very important bioactive properties. In the inflammatory phase of wound healing, following wound formation, PMNL migrates to the scar tissue in response to chemotactic molecules released from platelets.[5] Neutrophils are present in the wound area as the dominant cell for the first 2 days. These cells ensure that foreign bodies and bacteria are removed from the environment and phagocytosed.[6] The researchers state that these findings are related to the fact that chitosan stimulates the migration of cells that secrete growth factors and pro-inflammatory mediators in the early stages of recovery.[7]

Usami et al., in their study, they stated the importance of chitosan acting as a chemoattractant in PMNL increase. Usami et al.[8] again, in the same study, they showed that chitosan activates canine PMNL cells in vitro and increases cytokine production of canine PMNL cells directly or through complement activation in vivo.

Liu et al.[9] stated that chitosan-based hydrogels are effective in every phase of wound healing in their study to examine the effects of chitosan as a hydrogel wound dressing and on wound healing in drug delivery systems. They stated that chi-

tosan stimulates the formation of thrombosis and accelerates blood coagulation in vivo by triggering the activation of platelets, so the inflammatory phase begins in a shorter time. They stated that chitosan-based hydrogels provide a favorable environment for healing by regulating the relevant cells and factor release. In addition, they emphasized that chitosan increased the inflammatory properties of polymorphonuclear leukocytes, macrophages, and neutrophils and provided an appropriate inflammatory response in the study.

At the stage of acute inflammatory response with the arrival of neutrophils, monocytes/macrophages and mast cells to the wound area, these cells secrete inflammatory cytokines and growth factors that have important roles in wound healing. In their study, Okamoto et al.[10] emphasized that chitosan increases the release of platelet-derived growth factor-AB (PDGF-AB) and TGF-β1, which play an important role in wound healing.

Judith et al.[11] emphasized that chitosan increases the formation of PDGF in their study in which they examined the effects of collagen-chitosan matrix systems in rats in excision wounds. In the study, it was stated that chitosan accelerated the formation of granulation tissue due to increasing the formation of PDGF. They also stated that new epidermis tissue accompanied the capillaries formed in large numbers on the 10th day of excision in in vivo results.

In the histological findings of chitosan-treated wounds, when the experimental group and the control group were compared, it was reported that leukocytes and macrophages came to the healing area more quickly in the chitosan-treated group. These findings show that chitosan attracts inflammatory cells and growth factors released from these cells to the wound area in the early stages of wound healing. It has been stated that chitosan attracts VEGF, which is one of the growth factors released from the cells involved in the early phase of wound healing, to the healing area.[11]

As a result of our literature research, we did not find a study examining the effect of chitosan on wounds in the oral tissues of diabetic rats, so we compared the inflammation levels between the groups in this study. We found that there was a statistically significant difference between all groups in terms of the severity of inflammation on the 2nd day. As a result of the pairwise comparisons made to determine which group the difference originates from; We concluded that the difference was detected between the Control and Diabetes groups. When we look at the inflammation levels seen in the groups on the 2nd day, 20% of the experimental animals in the control group were mild; we observed moderate inflammation in 80% of them. In the Diabetes group, subjects had 100% severe inflammation and 37.5% moderate in the Diabetes + Chitosan group; we detected severe inflammation in 62.5%. When the inflammation rates between the groups were compared on the 2nd day, we observed that diabetes had a negative effect on inflammation. In addition, it was observed that chitosan had a positive effect on inflammation on the 2nd day of wound healing. When we looked at the inflammation levels seen in the groups on the 5th day, we found that there was a statistically significant difference between the groups. As a result of the pairwise comparisons made to determine which group the difference originates from; we found that there was a difference between the control group and the diabetes groups, and between the diabetes group and the Diabetes + Chitosan group. While the inflammation seen on the 5th day in the control group was 100% mild, 12.5% moderate in the Diabetes group; we observed severe inflammation in 87.5%, and 100% moderate inflammation in the Diabetes + Chitosan group. When the inflammation rates between the groups were compared on the 5th day, it was determined that diabetes had a negative effect on inflammation. In addition, the decrease in the severity of inflammation in the Diabetes + Chitosan group in our findings suggests that chitosan has a positive effect on inflammation on the 5th day of wound healing.

When the groups were evaluated in terms of fibrosis levels in our study, we found that there was no statistically significant difference between the groups on the 2nd day of wound healing. In the evaluation between groups, 20% of the experimental animals in the control group had no fibrosis on the 2nd day, while fibrosis was observed in 1–20% of the ulcer area in 80% of the experimental animals. In the diabetes group, 87.5% had no fibrosis, while 20% had fibrosis in 1–20% of the ulcer area. In the Diabetes + Chitosan group, 75% had no fibrosis, while 25% had fibrosis in 1–20% of the ulcer area. We found that there was a statistically significant difference between the groups in terms of fibrosis levels on the 5th day. In our 5th day findings, fibrosis was observed in 1–2% of the ulcer area in 20% of the experimental animals in the control group, while fibrosis was observed in 20–60% of the ulcer area in 80% of the experimental animals. While fibrosis was observed in 1–20% of the ulcer area in 50% of the diabetes group, fibrosis was observed in 20–60% of the ulcer area in the other 50% of the group. In the Diabetes + Chitosan group, fibrosis was observed in 1–20% of the ulcer area in 100% of the experimental animals. We observed that there was a decrease in fibrosis formation in the experimental group in which we applied chitosan, but this did not cause a significant difference with the diabetes group.

Although there was no significant difference in the diabetes group in our study, the reductions in the level of fibrosis observed on the 2nd and 5th days are compatible with studies that show that diabetes reduces fibrosis.

In our study, in the comparison between groups in terms of epithelial regeneration levels on day 2, more epithelial regeneration was observed in the control group than in other groups with diabetes. Epithelialization levels of the diabetes group and diabetes + chitosan group were found to be close to each other. In the comparison between the groups on the

5th day, a significant difference was found between the control group and the groups with DM, and it was observed that epithelial regeneration was more in the control group. No significant difference was found between the epithelization levels of the diabetes group and the diabetes + chitosan group. This difference in the control group on the 2nd and 5th days is thought to be due to the negative effect of diabetes on all stages of wound healing. Although chitosan is known to have an effect on tissue regeneration, it is thought that it does not affect wound healing through epithelial regeneration.

When the experimental animals in the groups in our study were evaluated in terms of necrosis, we could not find a statistically significant difference between the groups on the 2nd and 5th days of recovery. As a result of the values of control, diabetes and diabetes + chitosan groups that we found close to each other in percentage, we think that chitosan is not toxic and this does not have an effect on necrosis.

In our study, in which we examined the effect of chitosan on wound healing in wounds in the oral tissues of diabetic rats, it was shown that the healing processes of the wounds were positively affected. We believe that the findings we obtained in this study will contribute to the literature on the use of chitosan in diabetic patients in the clinic. However, our study was carried out on the oral mucosa, on which there is not much literature study. Therefore, there is a need for further studies on the use of chitosan in oral mucosal wounds in diabetic rats.

Conclusion

In this study model, in which we experimentally created DM;

A significant difference was found between the groups on the 2nd and 5th days of wound healing when compared in terms of inflammation levels. As a result of the decrease in inflammation levels in the chitosan-administered group, we observed that chitosan made a positive contribution to wound healing.

We determined that there was no significant difference between the groups in terms of fibrosis levels on the 2nd day of wound healing.

In the comparison of fibrosis levels between groups on the 5th day of wound healing; we observed a higher rate of fibrosis in the ulcerated area in the diabetes group compared to the diabetes+chitosan group.

We determined that chitosan had a slight positive effect on epithelial regeneration, but this effect was not statistically significant.

We determined that chitosan did not cause foreign body reaction in any of the experimental groups, so it was a biocompatible and non-toxic material.

In our study, we only made histopathological evaluations. We think that more effective results can be achieved with larger and more comprehensive studies in which histopathological and biochemical evaluations will be made together in the future.

Ethics Committee Approval: This study was approved by the İstanbul University Animal Experiment Ethics Committee (Date: 31.05.2018, Decision No: 2018/49).

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DENEYSEL ÇALIŞMA - *ÖZ*

Deneysel olarak oluşturulmuş diyabetik sıçanlarda kitosanın oral mukozada yara iyileşmesine etkisinin histopatolojik araştırılması

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AMAÇ: Çalışmamızda deneysel olarak oluşturulan diyabetes mellitusun jel formu uygulanarak kitosanın oral mukozada yara iyileşmesi üzerine etkilerinin histopatolojik olarak araştırılması amaçlandı.
GEREÇ VE YÖNTEM: Çalışmamızda 14–16 haftalık, 340±20 gram ağırlığında 42 adet Sprague Dawley cinsi erkek sıçan kullanıldı. Otuz iki olguya 55 mg/kg streptozotosin intraperitoneal (i.p.) uygulanarak diyabet indüksiyonu sağlandı. İkinci ve yedinci gün sonunda ölçülen kan şekeri 250 mg/dl'nin üzerinde olanlar diyabetik kabul edildi ve çalışmaya alındı. Daha sonra deney hayvanlarının bukkal mukozasında tek kullanımlık panç biyopsi aleti ile 5 mm çapında ve 1 mm derinliğinde yara oluşturuldu. Cerrahi operasyon sonrası ikinci ve beşinci günlerde yara iyileşmesi değerlendirildi. Örnekler histopatolojik olarak inflamasyon, fibrozis, epitel rejenerasyonu, nekroz ve yabancı cisim reaksiyonu açısından değerlendirildi.
BULGULAR: İstatistiksel analiz sonucunda ikinci ve beşinci gün enflamasyon düzeyleri açısından gruplar arasında anlamlı fark bulundu ($p<0.05$). Grup içi değerlendirmelerde diyabet+kitosan grubunda ikinci gün şiddetli enflamasyon oranı beşinci güne göre anlamlı derecede yüksek bulundu ($p<0.05$). İkinci gün fibrozis düzeyleri açısından gruplar arasında anlamlı fark bulunmazken ($p>0.05$), beşinci gün fibrozis düzeyleri açısından anlamlı fark bulundu ($p<0.05$).
TARTIŞMA: Kitosanın ikinci ve beşinci günlerde hiçbir grupta yabancı cisim reaksiyonuna neden olmadığı gözlendi.
Anahtar sözcükler: Histopatoloji; kitosan; yara.

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