TABLE 1 - TNF alpha and IL-6 levels and statistical intragoup comparison results.

	TNF alfa levels			IL-6 levels		
	Basal	24. hour	72. hour	Basal	24. hour	**72. hour**
Group S	38.3 ± 5.5	33.1± 1.1	34.6 ± 2.2	23.1 ± 4	19.3 ± 1.8	20.2 ± 3
Group K	36.1 ± 5.4	40.7 ± 4.9[a]	49 ± 4.3 [b, c]	24.6 ± 4.6	28.4 ± 5.7	51.5±13.2[b,c]
Group O	36.7 ± 5.6	38.3 ± 4.3	40.4 ± 2.7	23.1 ± 3.9	26.2 ± 5.1	31 ± 11

a:p<0.05 between basal to 24. hour, b:p<0.05 between basal to 72. hour, c:p<0.05 between 24. to 72. hour. Data are described as means ± standard deviations.

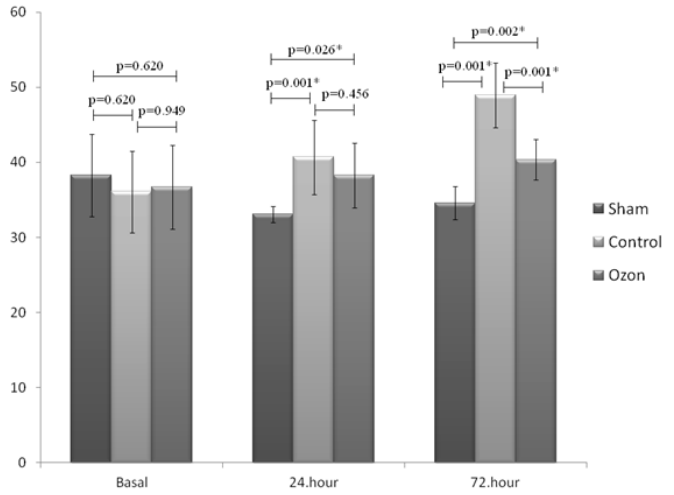

FIGURE 1 - Graphical representation of TNF alpha levels and statistical intergoup comparison results. Data are described as means ± standard deviations.

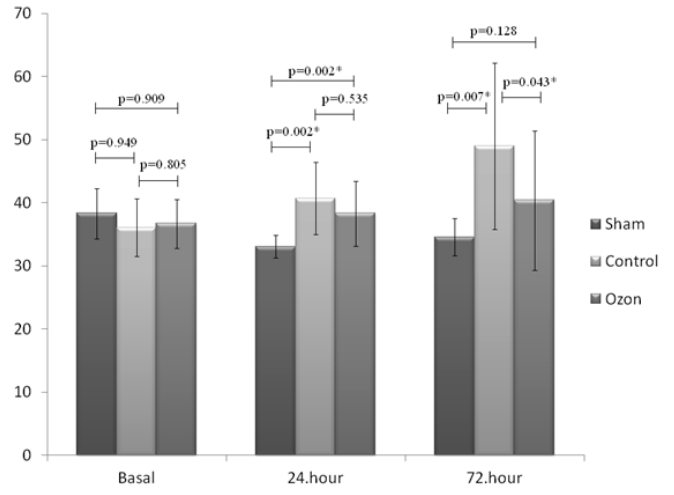

FIGURE 2 - Graphical representation of IL-6 levels and statistical intergoup comparison results. Data are described as means ± standard deviations.

IL-6: There was no statistically significant difference between the groups in comparison to basal values in all three groups (p>0.05). Any significant change was not observed in Group S in the intragroup assessments. The increase at the 24th hour was not significant compared with basal values in Group K (p=0.176). When the values measured at basal-72nd hour (p=0.043) and 24th hour-72nd hour(p=0.033) were compared, asignificant increase was observed. There was not a significant increase at 24th hour (p=0.499), 72nd hour (p=0.310), and between 24th-72th hours (p=0.469) compared with basal values in Group O. There was no significant difference between control and ozone groups (p>0.05) while a significant increase at the intergroup 24th hour in both control and ozone groups compared with Sham group. At the intergroup 72nd hour, there was not a significant difference between Sham-ozone and control-ozone groups (p>0.05) while there was a significant difference between Sham and control groups (p<0.05) (Table 1, Figure 2).

Histopathological examination

Tissue samples had the normal histological appearance and the cells on layers were easily distinguished by morphological features in Group S (Figure 3, Table 2). Increased angiogenesis and vasculogenesis, along with the regeneration of the epidermal and dermal, prominently increasing wound thickness due to the formation of granulation tissue, the indistinct cells located in the upper epidermal layer were observed in Group K. Though the limits of Squamous epithelial layers are not certain, it was determined that re-epithelialization increased due to this the edema areas were taken shape between the connective tissue in the dermal layer. It was seen that inflammatory cell infiltrations increased in areas close to the veins, the cells formed groups in such a way that forming clusters from place to place (Figure 4, Table 2).

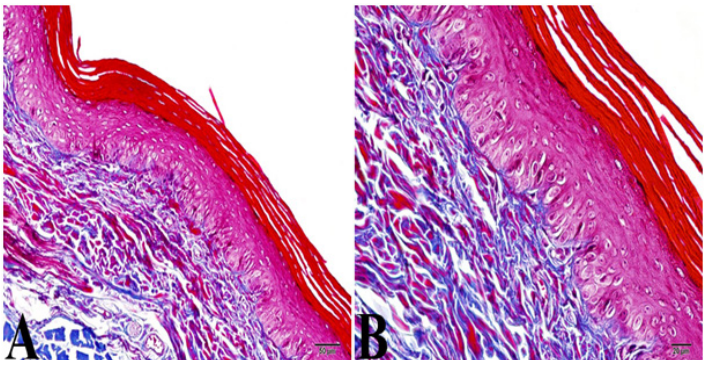

FİGURE 3 - Sham group. Masson Trichrome staining. Normal tissue appearance.

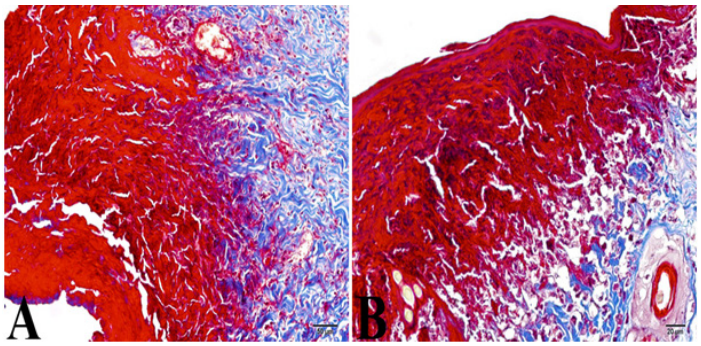

FİGURE 4 - Control group. Masson Trichrome staining. Histopathological appearance consistent with significant tissue injury.

TABLE 2 - Histopathological grading of all groups.

Group	Granülasyon doku kalınlaşması	Epidermal reje-nerasyon	Dermal rejenerasyon	Kollajen dağılımı	Angiogenesis	İnflamatuar hücreler
Grup S	0.00±0.42	1.00±0.48	1.00±0.52	1.00±0.00	0.00±0.97	0.00±0.42
Grup K	2.00±0.52[a]	2.00±0.00[a]	2.00±0.42[a]	2.00±0.70[a]	2.00±0.67[a]	3.50±0.69[a]
Grup O	3.00±0.32[a,b]	3.00±0.00[a,b]	3.00±0.32[a,b]	4.00±0.48[a,b]	3.00±0.57[a,b]	2.00±0.00[a,b]

[a]:A significant difference was found in the statistics done in terms of histopathological changes such as granulation tissue thickness, epidermal regeneration, dermal regeneration, collagen distribution, angiogenesis, inflammatory cells according to Mann-Whitney U test done between Group S, Group K, and Group O ($p<0.05$).
[b]:A significant difference was found in the statistics done in terms of histopathological changes such as granulation tissue thickness, epidermal regeneration, dermal regeneration, collagen distribution, angiogenesis, inflammatory cells according to Mann-Whitney U test done between Group K and Group O ($p<0.05$).

It was seen that in Group O, angiogenesis and vasculogenesis increased more, these areas were found to be better identified with especially Mason staining. It was observed that granulation tissue was prominently formed together with epidermal and dermal regeneration, the thickness of the wound was less than Group K. It was determined that especially the cells located in the upper part of the epidermal layer became distinct and keratinisation on stratum korneum emerged. Though the limits of Squamous epithelial layers are not certain, it was seen that the borders of the cell could be identified and angiogenesis, vasculogenesis, and re-epithelialization markedly increased compared to Group K.

Despite the edema areas between the connective tissue in the dermal layer are similar to Group K, it was observed that the thickness of collagen threads increased and collagen deposition was higher in this group. It was seen that inflammatory cell infiltrations were in areas close to the veins, but the cells were in a single view in a manner that not to form groups (Figure 5, Table 2). It was observed that the wound healing process proceeded more slowly in the control group. It was determined that the wound healing was prominently accelarated in Ozone group.

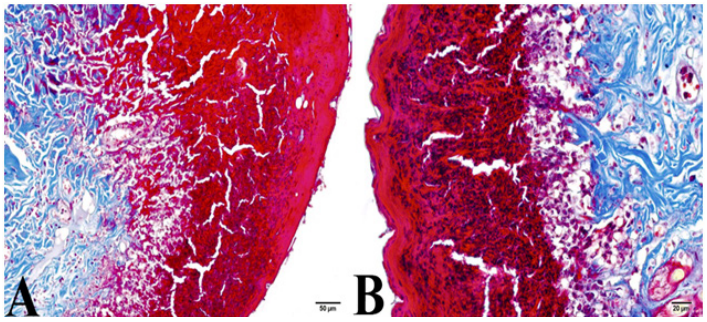

FİGURE 5 - Ozone group. Masson Trichrome staining. Histopathological appearance consistent with better wound healing process.

Discussion

The decrease was observed in the proinflammatory cytokines; TNF alpha and IL-6 in the ozone treated group in our study. It was seen that the prominent formation of granulation tissue along with the regeneration of the epidermal and dermal, the wound thickness was less, angiogenesis, vasculogenesis, and re-epithelialization intensely increased in the histopathological examination. It was observed that the thickness of collagen tissue

increased and collagen deposition was higher in this group and wound healing was prominently faster.

Ozone is a molecule which affects systemically[5]. It triggers reactions that affecting each other, when it is taken into the body. The increase in antioxidant level and the reduction in lipid peroxidation occur at the end of these reactions. This effect reverts in a short time. Ozone also activates "heme oxygenase 1" enzyme, too. This enzyme has antioxidant, antiapoptotic, and antiinflammatory effects[11].

It is reported that many factors have effects on wound healing[12,13]. There are studies investigating the effects of ozone treatment on infected wound healing. Lelyanov et al. for the first time, investigated the effects of ozone on wound healing[14]. In that study, it was reported that ozone made a positive contribution to the healing of colonic anastomosis wound. Again, Erginel et al. looked for the effect of ozone on colonic anastomosis wound healing in a rat model of peritonitis and stated that wound healing was better in the ozone treated groups, in their study[15]. It was determined that topical application of ozone had healing effects on acute skin wounds[16]. We observed that ozone had healing effects on surgical wounds of rabbits that we applied prophylactic ozone postoperatively in our study. Wound healing was found to be prominently accelerated in the rabbits treated with ozone. When we looked through in terms of histopathological changes such as granulation tissue thickening, epidermal regeneration, dermal regeneration and collagen distribution, angiogenesis, inflammatory cells, we observed that ozone had positive effects on wound healing at the same time.

Many studies have reported that various cell sources contribute to the production of cytokines in wound1[7,18]. Proinflammatory cytokins; TNF alpha and IL-6 have negative effects on wound healing[19]. The decrease on levels of these cytokins will create positive impact on wound healing. We evaluated the antiinflammatory effects through these mediators in our study. Though there was not statistically significant decrease in IL-6 and TNF alpha overall values, it was found to be lower in ozone treated group compared with control group.

In the histopathological examination in our study, the decrease in the number of anti-inflammatory cells and also reduction of proinflammatory cytokine levels suggested that antiinflammatory activity of ozone had a positive effect on wound healing. Especially the increment of the cells located in the upper part of the epidermal layer in ozone treated group compared to control group was consistent with the reduction of cell apoptosis. Though the edema areas between connective tissue in the dermal layer was similar to control group, collagen deposition was higher in the ones treated with ozone made us think that ozone made a positive contribution to wound healing with its antioxidant property.

Kim *et al*.[16] reported that the sizes of wound were small, collagen fibers and fibroblasts prominently increased in the ozone treated group in their study of local application of ozone on acute cutaneus wound healing. It was shown that reduction of wound size and the significant increase in collagen fibers after ozone treatment on diabetic wound[20]. These results suggest us the positive effect on wound healing of the ozone might be associated with its antiinflammatory, antiapoptotic, and antioxidant properties.

Conclusion

Preoperative rectal application of ozone had positive effects on wound healing in acute period.

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Erratum

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DOI: http://dx.doi.org/10.1590/S0102-865020160070000007

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TABLE 2 - Histopathological grading of all groups.

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Grup S	0.00±0.42	1.00±0.48	1.00±0.52	1.00±0.00	0.00±0.97	0.00±0.42
Grup K	2.00±0.52[a]	2.00±0.00 [a]	2.00±0.42 [a]	2.00±0.70 [a]	2.00±0.67 [a]	3.50±0.69 [a]
Grup O	3.00±0.32 [a,b]	3.00±0.00 [a,b]	3.00±0.32 [a,b]	4.00±0.48[a,b]	3.00±0.57 [a,b]	2.00±0.00 [a,b]

[a]:A significant difference was found in the statistics done in terms of histopathological changes such as granulation tissue thickness, epidermal regeneration, dermal regeneration, collagen distribution, angiogenesis, inflammatory cells according to Mann-Whitney U test done between Group S, Group K, and Group O ($p<0.05$).

[b]:A significant difference was found in the statistics done in terms of histopathological changes such as granulation tissue thickness, epidermal regeneration, dermal regeneration, collagen distribution, angiogenesis, inflammatory cells according to Mann-Whitney U test done between Group K and Group O ($p<0.05$).

Consider this Table 2:

TABLE 2 - Histopathological grading of all groups.

Group	Granulation Tissue Thickening	Epidermal Regeneration	Dermal Regeneration	Collagen Distribution	Angiogenesis	Inflammatory Cells
Grup S	0.00±0.42	1.00±0.48	1.00±0.52	1.00±0.00	0.00±0.97	0.00±0.42
Grup K	2.00±0.52[a]	2.00±0.00 [a]	2.00±0.42 [a]	2.00±0.70 [a]	2.00±0.67 [a]	3.50±0.69 [a]
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