doi: http://dx.doi.org/10.22201/fmvz.24486760e.2024.1203
Vol. 11 | 2024

Veterinaria México OA
Publicación Digital de la Facultad de Medicina Veterinaria y Zootecnia https://veterinariamexico.fmvz.unam.mx/

Efficiency of ozone (O_3) therapy on experimental acidic skin burns in rats

Nevzat Emre Aslan[1]
0000-0001-8970-7763
Hanifi Erol[2,*]
0000-0001-8140-3108
Esra Balcıoğlu[3]
0000-0003-1474-0432
Betül Yalçın[4]
0000-0003-1176-8843

[1] University of Bozok. Faculty of Veterinary Medicine. Department of Surgery. Yozgat, Turkey.

[2] University of Erciyes. Faculty of Veterinary Medicine. Department of Surgery. Kayseri, Turkey.

[3] Erciyes University. Faculty of Medicine. Department of Histology and Embryology. Kayseri, Turkey.

[4] Adıyaman University. Faculty of Medicine. Department of Histology and Embryology. Adıyaman, Turkey.

***Corresponding author**
Email address:
drhaneroll@yahoo.com

Abstract

In this study, it was aimed to investigate the efficacy of O_3 therapy in experimentally induced hydrofluoric acid (HF) skin burns in rats. A total of 20 healthy male Wistar Albino rats (weighing 250-300 g, aged 16 weeks) were used as the material of the study. They were divided into two groups (as experimental and control groups) of 10 rats which were housed individually and fed *ad libitum*. HF skin burns were induced in all animals. The ozonized (20 µg O_3/mL) liquid vaseline was applied topically for seven days to the experiment group as well as parallelly, a saline solution was applied to the control group. In the histopathologic evaluation, inflammation, vascularization, epithelial regeneration and fibrosis were evaluated. The epithelial proliferation and collagenization were higher and statistically significant in the experimental group, while the infiltration was higher and statistically significant in the control group. As a result of this study, it was observed that ozone plays an important role in the tissue repair process in chemical burns. The increase of fibroblast activation and re-epithelization in the experimental group demonstrated that ozone therapy could be suggested as first aid in HF skin burns. It was concluded that more studies on ozone are needed to shed light on the subject.

Keywords: Hydrofluoric acid (HF); Ozone therapy; Wistar Albino rats; Fibroblast activation; Chemical burns.

Submitted: 2023-04-17
Accepted: 2023-09-01
Published: 2024-02-14

Additional information and declarations can be found on page 9

Cite this as:

Aslan NE, Erol H, Balcıoğlu E, Yalçın B. Efficiency of ozone (O_3) therapy on experimental acidic skin burns in rats. Veterinaria Mexico OA. 2024;11(23). doi: 10.22201/fmvz.24486760e.2024.1203.

doi: http://dx.doi.org/10.22201/fmvz.24486760e.2024.1203
Vol. 11 | 2024

Study contribution

Due to industrial development associated changes in environmental conditions, contact risk to chemical agents increases in animals. Therefore, chemical contact burn is high and common in animals. Ozone is a medical gas and used for clinical treatment in recent years, proper different concentration to improve oxygen supply in local tissue, inhibit inflammatory and stress factors. This study aimed to investigate the efficacy of ozone therapy in experimentally induced hydrofluoric skin burns. The results demonstrated that ozone therapy could be a viable option for treating acidic skin burn. Ozone repairs degenerated tissues by increasing fibroblast activation and promoting re-epithelization.

Introduction

Burns are still a frequent trauma worldwide. With the change in the environmental conditions due to industrial development, the risk of contact with chemical agents and, therefore, the risk of chemical burns increases both in humans and animals. Numerous experimental studies are still being conducted on burn treatments.[1] Hydrofluoric acid (HF) is a chemical compound widely used in the industry as a metal and glass cleaner. HF skin burns are physiopathologically different from other acid burns. The hydrogen ions of HF have a corrosive effect on the skin, allowing the fluoride ions to enter the circulatory system through the skin, where they cause poisoning by binding the calcium and magnesium present in the serum. Also, the release of potassium from red blood cells is increased, producing liquefaction necrosis and bone decalcification in deep tissues. Due to the mentioned effects, the skin burn induced by HF causes severe pain and a systemic toxic effect.[2]

Ozone (O_3) is widely used for medical purposes as antibacterial, antiviral, antifungal and for healing wounds.[3] The systemic effects of O_3 are the increase of blood flow and oxygen transfer in ischemic tissues, the release of growth factors by the activation of the immune system, the disinfection when topically applied and the stimulation of the neuroendocrine system. It is especially the positive effect on the transportation and release of oxygen to the tissues, the preferred feature for the treatment of different wound types.[4,5]

Despite the fact that HF is extensively used in the industry and that related skin burns are frequently encountered, in the literature reviews, no study has been found on the investigation of the therapeutic effects of O_3 in HF skin burns. In this study, it was aimed to investigate the efficacy of O_3 therapy in experimentally induced HF skin burns in rats.

Materials and methods

Ethical statement

The experimental protocol was approved by the Animal Ethical Committee of the University of Erciyes (approval number: 21/28).

doi: http://dx.doi.org/10.22201/fmvz.24486760e.2024.1203
Vol. 11 | 2024

Animal grouping

The study was conducted on a total of 20 healthy male Wistar Albino rats (weighing 250-300 g, aged 16 weeks), which were divided into two groups (experimental and control) of 10 rats housed individually and fed ad libitum.

Anaesthesia and experimental HF burn

After routine examination, for general anaesthesia, xylazine [5 mg(kg)$^{-1}$] and ketamine [60 mg(kg)$^{-1}$] were applied intraperitoneally. All animals were positioned dorso-ventrally, and a thoracolumbar skin region of 3 × 2 cm^2 was prepared for HF burn. The modified experimental procedure of Cavallini and Casati was used to induce skin burn. 0.1 mL HF (38 % Merck, USA) was dropped on the skin and left for 2 min to produce the burn.[6] After that time, the area was washed with saline solution for 3 min, after which Doxycycline [5 mg(kg)$^{-1}$] (Peradoks 100 mg/mL, Alke, Tokat, Turkey) for 5 days and Calpol [2 mg (kg)$^{-1}$] (Paracetamol, 24 mg/mL, GlaxoSmith-Kline, Turkey) for 2 days were administered orally as an analgesic and to avoid the risk of secondary infection, respectively.

Ozone administration

The ozone generator used was (Evozone Basic Plus GMBH Germany) adjusted to 80 µg/mL for O_3 concentration and fed with pure oxygen at a flow rate of 500 mL/min. The liquid vaseline (20 mL) was ozonized for 10 min at 20 °C, and the maximum O_3 concentration was obtained. The ozonized (20 µg O_3/mL) for liquid vaseline was applied topically for seven days to the experiment group, while, parallelly, a saline solution was applied to the control group. The ozonization procedure for liquid vaseline was repeated before each application in order to apply the same amount of O_3. All animals were sacrificed under general anaesthesia on the 8th day, the last day of application.

Histopathological evaluations

The skin samples obtained from the experimental and control groups were fixed in 10 % formaldehyde solution for 48 h. After the fixation process, the tissues were washed in tap water and passed through graded alcohol series. Then the tissues were cleared with xylol and embedded in paraffin, and the blocking process was performed. Five micrometres thickness sections were taken from paraffin blocks, then cleaned with xylene and dehydrated by passing through graded alcohol series. The sections were stained with Hematoxylin&Eosin (H&E) and Masson's trichrome (MT) and evaluated according to the histopathological scoring parameters under the Olympus BX51 (Japan) light microscope. The criteria set for evaluation are as follows: epithelial proliferation, collagenization and infiltration (0: no detection, 1: poor detection, 2: intermediate detection, 3: prominent detection).

Statistical analysis

Statistical Package for Social Sciences (SPSS) 22.0 for Windows was used for statistical analysis. The results of the study were shown as mean ± standard deviation (X ± SD),

doi: http://dx.doi.org/10.22201/fmvz.24486760e.2024.1203
Vol. 11 ▮ 2024

and P values below 0.05 were considered statistically significant. Shapiro-Wilk test was used for the normality of values. T-test was used for comparisons between groups.

Results

After the application of HF, redness, erythema and white vesicles were seen macroscopically in all animals. The macroscopic findings showed 2nd degree burns in the skin. The clinical picture started to turn normal on the 3th day of applying O_3 to the experimental group. In contrast, the clinical findings started to turn into local necrosis and crusted wounds in the same period in the control group. On the 8th day, seven animals in the experimental group and three animals in the control group were seen macroscopically completely recovered. In the histopathologic evaluation, inflammation, vascularization, epithelial regeneration and fibrosis were evaluated at 4× and 10× in sections stained with H&E and MT in the control group, irregular dermal wound areas, cell loss of the stratified squamous epithelium and low re-epithelialization were detected.

In addition, some keratinocyte migration on the stratified epithelial tissue in the burned area and scar tissue were distinguished. The presence of scabs in this area was a sign of serious damage and delayed healing in the control group. In some areas, a thin layer formed by mitotic cells was seen in the stratum basale, and an irregular dermis layer was also observed. The dense infiltration and enlarged blood vessels were seen beneath the connective tissue of the burned areas. In addition, foci of haemorrhage with collagen loss and angiogenesis formation were also detected in the general structure. In the necrotic area of the dermis under the burned region, no hair follicle or sebaceous gland was found (Figure 1).

In the experimental group, an increased cell loss on stratified epithelial tissue and a significant increase in re-epithelialization were detected. Contrary to the control group, keratinocyte migration and scab were seldom seen in the experimental group. The shrinkage of the scab on the ozonized area showed that the healing in the burn area was faster than in the control group. It was determined that the cells in the stratum basale layer formed by mitosis stratum spinosum, stratum granulosum, stratum lucidum, and even stratum corneum layers. A decrease was observed in the areas of irregularity and ulceration and neutrophil infiltration in the dermis layer.

An increase was observed in the amount of collagen compared to the control group. There were also reduced foci of haemorrhage, fibrosis, and necrotic areas with fewer dilated blood vessels (Figure 2). The hair follicles and sebaceous glands were prominent under the burn area. The statistical comparison of epithelial proliferation, collagenization and infiltration between groups is shown in Table 1. The epithelial proliferation and collagenization were higher and statistically significant in the experimental group, while the infiltration was higher and statistically significant in the control group.

doi: http://dx.doi.org/10.22201/fmvz.24486760e.2024.1203
Vol. 11 | 2024

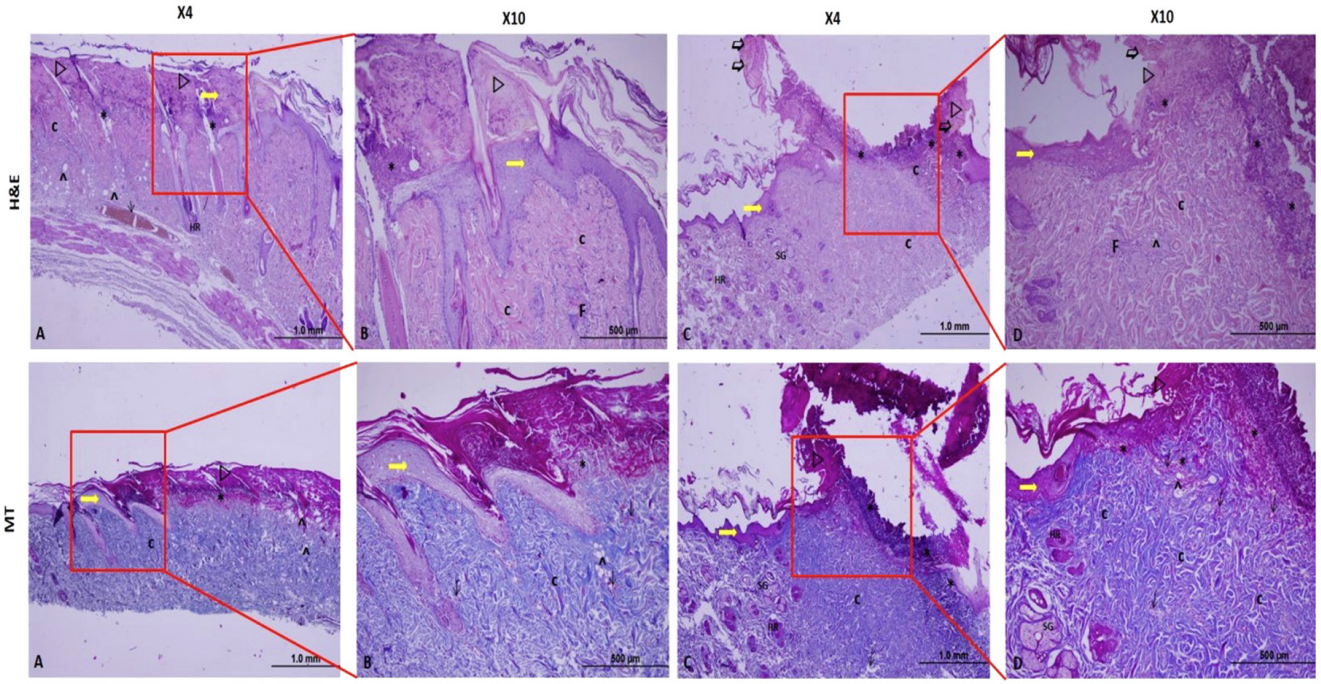

Figure 1. General view of the wound area of the control group. ➔: Stratified squamous epithelium, C; Collagen,→; Enlarged blood vessels, HR; Hair root, ^; lymphovascular structure, ▷; Scab, *; Infiltration, F; Fibroblast proliferation, SG; Sebaceous gland, ⇨;Bleeding.

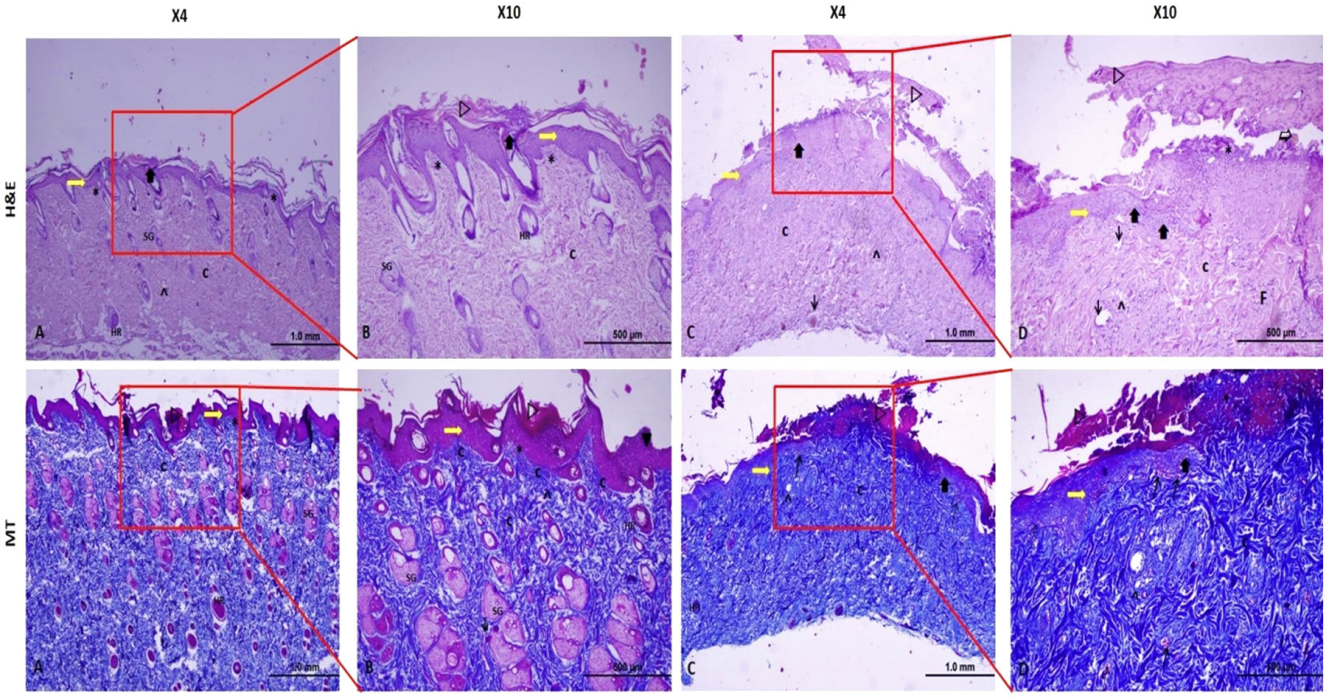

Figure 2. General view of the wound area of the experimental group. ➔: Stratified squamous epithelium, C; Collagen, →; Enlarged blood vessels, HR; Hair root, ^; lymphovascular structure, ▷; Scab, *; Infiltration, F; Fibroblast proliferation, SG; Sebaceous gland, ⇨; Bleeding, ⬆; Reepithelialization.

doi: http://dx.doi.org/10.22201/fmvz.24486760e.2024.1203
Vol. 11 | 2024

Table 1. Epithelial proliferation, collagenization and infiltration scores of the control and experimental groups

	Control	Experimental	P
Epithelial proliferation	0.30 ±0.48[a]	1.80 ±1.13[b]	0.0010
Collagenization	0.70 ±0.67[a]	1.90 ±0.87[b]	0.0030
Infiltration	2.20 ±0.78[a]	0.50 ±0.52[b]	0.0140

The same letters on the same line indicate the similarity between the groups, and different letters indicate the difference.

Discussion

It has been reported that HF burns are physiopathologically different from other acidic burns due to the rapid access of highly permeable fluoride ions to deep tissues. Different treatment methods have been suggested for HF burns, and the primary goals of these methods are to prevent damage to deep tissue by disrupting the effects of fluoride ions.[2,7] Ozone is used medically for the treatment of many diseases such as anaerobic infections, abscesses, decubitus wounds, fistulas, skin wounds and gingivitis.[8,9] It stimulates the release of Hydrogen Peroxide from the immune system cells and activates the immune system.

Furthermore, it causes serious damage to the cell wall of viral and bacterial pathogens by oxidizing the phospholipids of the pathogens along with the stimulation of growth factors.[4,5,10] In the literature review, no study was found on the effectiveness of O_3 in treating HF burn. For this reason, it was aimed to investigate the effectiveness of O_3 in experimental HF skin burn treatment and therefore contribute to clinical practice and literature. During the healing period of a skin wound, hemopoietic, endothelial, neural cells and growth factors play important roles; nevertheless, it has been stated that keratinocytes and fibroblasts have the most important role in the healing process by migrating to the wound area. Additionality, fibroblasts have been reported to modulate immune cell functions by mediating fibrosis.[11]

Roblin et al. induced HF skin burns in rats and applied 2.5 % calcium gluconate gel topically for 17 days. At the end of that study, it was suggested that 2.5 % calcium gluconate should be used as first aid in HF burn wounds.[12] In another study, Kodik et al. induced HF skin burns in rats and applied epithelial growth factor subcutaneously. They emphasized that epithelial growth factor could be used in cases of HF skin burn for first aid, but its obtention is difficult and expensive. Therefore, they suggested using magnesium sulfate and calcium gluconate instead of epithelial growth factor.[13] For the treatment of HF skin burn, the researchers focused on the binding of magnesium and calcium by HF.[12, 13]

Researchers agree that after correcting hypomagnesemia and hypocalcemia, the cardiovascular and circulatory systems return to normality, and then recovery occurs. In the present study, the medical effects and therapeutic properties of O_3 were investigated in HF skin burns.[12,13] Data show that O_3 has potentially positive effects on the healing of HF skin burn. The epithelial proliferation and collagenization in the experimental group supported this condition. Pchepiorka et al. created surgical wounds on the buccal mucosa of rats and used O_3 for the treatment. It was found that the collagen fibres accumulated widely in the ozone group, whereas infiltration was more intense in the control group.

doi: http://dx.doi.org/10.22201/fmvz.24486760e.2024.1203
Vol. 11 | 2024

This result was attributed to O_3 effects such as angiogenesis, increased tissue oxygen, and increased expression of cytokines and growth factors.[14] Guven et al. created caustic oesophagal burn and used O_3 therapy in rats. They found that O_3 therapy has a beneficial effect on the healing process in caustic esophagitis by improving antioxidant defence mechanisms and reducing tissue damage, and ameliorating histopathological injuries.[8] Similar results were observed in the present study. Especially the decrease in neutrophil infiltration, the increase of collagen fibres and epithelial proliferation in the experimental group, along with the intensive infiltration in the control group, supported the O_3 effects on healing.

Ozone therapy has been used to accelerate the healing process, reducing inflammatory response and oedema. There are different O_3 therapy routes such as gas, ozonized oil and ozonized water that could be used for treatment.[15] Kim et al. reported that the topical use of ozonized oil was effective in increasing wound healing in the epithelium of rats, promoting the proliferation and synthesis of collagen on injury sites, and increasing growth factors expression such as PDGF, TGF-β and VEGF.[16] In this study, ozonized liquid vaseline was used topically for HF skin burn in the experimental group, and the recovery was found to be better and faster than in the control group. Therefore, this study showed that ozonized liquid vaseline could be used topically in HF skin burn and obtained similar results to related studies.[15,16]

The concentration, dose and application frequency of O_3 are essential for the treatment. The standardization of the O_3 dose is not clearly revealed in animals. In humans, a dose of 27–50 μg O_3/mL O_3 was reported for satisfactory results. It is reported that 60 μg O_3/mL improves the effects without producing toxicity and accelerates the healing process.[14] Concurrently, some researchers emphasized that the size and width of the lesion affect the dose of O_3 needed for the treatment. Some studies have developed methodologies with greater lesions and more sessions of O_3 application.[16,17] In the present study, 20 μg O_3/mL was used daily in the experimental group, and it was observed that 20 μg O_3/mL was adequate for the treatment. However, even though the use of 20 μg O_3 in the study was sufficient for the treatment, the size of the lesion created, the width, and the blood supply of the tissue could affect the treatment.

This condition may cause changes in the dose of O_3. During the period of healing of a chemical skin burn, the degree of burn, the timing, and the management of the treatment affect the chances of success of the treatment. The risks of infection and systemic complications could be seen in this period. Especially, inflammatory processes in the epidermis, dermis and subcutaneous tissue can lead to more serious problems.[18] In the present study, no infection or complication was detected. Although it is stated that O_3 has an antimicrobial effect, it should not be ignored that no infection has occurred due to the use of antibiotic in the study. On the other hand, compared with previous studies, it was observed that O_3 accelerated and shortened the healing process. This statement was supported by the detection of healing activity on a cellular basis.[12-14]

doi: http://dx.doi.org/10.22201/fmvz.24486760e.2024.1203
Vol. 11 | 2024

Conclusion

As a result of this study, it was observed that O_3 plays an important role in the tissue repair process in chemical burns. The increase of fibroblast activation and re-epithelization in the experimental group demonstrated that O_3 therapy could be suggested as first aid in HF skin burn. It was concluded that more ozone-related studies should be done to enlighten the subject.

doi: http://dx.doi.org/10.22201/fmvz.24486760e.2024.1203
Vol. 11 | 2024

Data availability

All relevant data are within the manuscript and its supporting information files.

Funding statement

This study was supported by Erciyes University, Scientific Research Projects Coordination Unit with the project number TYL-11042.The funder had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Conflicts of interest

The authors declared that there is no conflict of interest.

Author contributions

Data curation: Hanifi EROL, Nevzat Emre ASLAN
Formal analysis: Hanifi EROL, Esra BALCIOGLU
Funding acquisition: Hanifi EROL, Nevzat Emre ASLAN
Investigation: Hanifi EROL, Nevzat Emre ASLAN
Methodology: Hanifi EROL, Nevzat Emre ASLAN, Esra BALCIOGLU, Betül YALÇIN
Project administration: Hanifi EROL
Resources: Hanifi EROL, Nevzat Emre ASLAN
Software: Hanifi EROL, Nevzat Emre ASLAN
Supervision: Hanifi EROL
Validation: Hanifi EROL
Visualization: Esra BALCIOGLU, Betül YALÇIN
Writing-original draft: Nevzat Emre ASLAN, Hanifi EROL, Esra BALCIOGLU, Betül YALÇIN
Writing-review and editing: Hanifi EROL, Esra BALCIOGLU

References

1. Arda MS, Kuas N, Söztutar E, Koçman AE, İlhan H. Comparison of burn depth at different temperatures on ex vivo human skin with standardized model and comparison of the results with rat contact burn model. Pediatric Research. 2018;5(2):88-91. doi: 10.4274/jpr.47113.
2. McKee D, Thoma A, Bailey K, Fish Z. A review of hydrofluoric acid burn management. Plastic Surgery. 2014;22(2):95-98. doi: 10.1177/229255031402200202.
3. Üstebay S, Öztürk Ö, Bilge A, Üstebay DÜ, Tezcan AH. Impacts of ozone treatment and its relationship with IGF-1 levels after injury of soft tissue: an experimental study in rats model. Kafkas Universitesi Veteriner Fakültesi Dergisi. 2017;23(6):967-971. doi: 10.9775/kvfd.2017.18058.
4. Fitzpatrick E, Holland OJ, Vanderlelie JJ. Ozone therapy for the treatment of chronic wounds: a systematic review. International Wound Journal. 2018;15(4):633-644. doi: 10.1111/iwj.12907.
5. Pivotto AP, Banhuk FW, Staffen IV, Daga MA, Ayala TS, Menolli RA. Clinical uses and molecular aspects of ozone therapy: a review. Online Journal of Biological Sciences. 2020;20(1):37-49. doi: 10.3844/ojbsci.2020.37.49.
6. Cavallini M, Casati A. A prospective, randomized, blind comparison between saline, calcium glukonate and diphoterine for washing skin acid injuries in

doi: http://dx.doi.org/10.22201/fmvz.24486760e.2024.1203
Vol. 11 | 2024

rats: effects on substance p and β endorphin release. European Journal of Anesthesiology. 2004;21:389-392. doi: 10.1017/S0265021504005071.

7. Saracoglu A, Kuzucuoglu T, Yakupoglu S, Tuncay E, Demirhan R. Chemical burn injury due to hydrofluoric acid: a case of successful management without hexafluorine. Marmara Medical Journal. 2015;28:161-164. doi: 10.5472/MMJcr.2803.02.
8. Guven A, Gundogdu G, Sadir S, Topal T, Erdogan E, Korkmaz A, Surer I, Ozturk H. The efficacy of ozone therapyin experimental caustic esophageal burn. Journal of Pediatric Surgery. 2008;43:1679-1684. doi: 10.1016/j.jpedsurg.2008.01.064.
9. Valacchi G, Fortino V, Bocci V. The dual action of ozone on the skin. British Journal of Dermatology. 2005;153(6):1096-1100. doi: 10.1111/j.1365-2133.2005.06939.x.
10. Zeng J, Lu J. Mechanisms of action involved in ozone-therapy in skin diseases. International Immunopharmacology. 2018;56(138):235-241. doi: 10.1016/j.intimp.2018.01.040.
11. Rippa AL, Kalabusheva EP, Varotelyak EA. Regeneration of dermis: scarring and cells involved. Cells. 2019;8(6):607. doi: 10.3390/cells8060607.
12. Roblin I, Urban M, Flicoteau D, Martin C, Pradeau D. Topical treatment of experimental hydrofluoric acid skin burns by 2.5% calcium gluconate. Journal of Burn Care & Research. 2006;27(6):889-894. doi: 10.1097/01.BCR.0000245767.54278.09.
13. Kodik MS, Akdemir O, Lineawear WC, Cavusoglu T, Ozsarac M, Aktug H, Songur E, Tiftikoglu YO. Comparison of skin effects of immediate treatment modalities in experimentally induced hydrofluoric acid skin burns. International Wound Journal. 2015; 12(6):716-723. doi: 10.1111/iwj.12214.
14. Pchepiorka R, Moreira MS, Lascane NA, Catalani LH, Allegrini S, Lima NB, Gonçalves F. Effect of ozone therapy on wound healing in the buccal mucosa of rats. Archives of Oral Biology. 2020;119(1):1-7. doi: 10.1016/j.archoralbio.2020.104889.
15. Naik SV, Rajeshwari K, Kohli S. Ozone-a biological therapy indentistry-reality or myth? The Open Dentistry Journal. 2016;10(1):196-206. doi: 10.2174/1874210601610010196.
16. Kim HS, Noh SU, Han YW, Kim KM, Kang H, Kim HO, Park YM. Therapeutic effects of topical application of ozone on acute cutaneous wound healing. Journal of Korean Medical Science. 2009;24:368-374. doi: 10.3346/jkms.2009.24.3.368.
17. Ripamonti CI, Maniezzo M, Boldini S, Pessi MA, Mariani L, Cislaghi E. Efficacy and tolerability of medical ozone gas insufflations in patients with osteonecrosis of the jaw treated with bisphosphonates preliminary data medical ozone gas insufflation in treating onj lesions. Journal of Bone Oncology. 2012;1:81-87. doi: 10.1016/j.jbo.2012.08.001.
18. Diaz JH, Lopez FA. Skin, soft tissue and systemic bacterial infections following aquatic injuries and exposures. The American Journal of the Medical Sciences. 2015;349(3):269-275. doi: 10.1097/MAJ.0000000000000366.