Journal of Tissue Viability 30 (2021) 196–206

Contents lists available at ScienceDirect

Journal of Tissue Viability

journal homepage: www.elsevier.com/locate/jtv

Comparison of the efficacy of multiple antioxidant and hyperbaric oxygen treatments in the prevention of ischemia and necrosis of local random McFarlane skin flap

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ARTICLE INFO

Keywords:
Multiple antioxidant
Ischemia
Skin flap
Survival
Rat

ABSTRACT

Objective: The aim of this study was to compare the efficacy of multiple antioxidant (Proxeed Plus (PP) with Carnitine, Selenium, Zinc, Coenzyme Q10, Vitamin C, Folic Acid, Vitamin B12) on local random skin flap healing with the hyperbaric oxygen (HBO) therapy.
Methods: Fourty rats were equally divided into five groups (Control, PP, HBO, HBO + PP, PP + HBO + PP). Local random McFarlane skin flap was applied to all rats.
Following the applications, evaluations were made biochemical (TAS, TOS, OSI, IL-1β, IL-6, TNF-α, TGF-β, VEGF) and histopathological parameters.
Results: Necrosis percentage was found to be lower in the PP + HBO + PP group than all other groups whereas the necrosis percentages of PP and HBO groups were similar. Oxidative stress rates were significantly higher in the control group compared to the other groups whereas it was lower in the PP + HBO + PP group than all other groups. The inflammation parameters were the highest in the control group and the lowest in the PP + HBO + PP group. Growth factors were higher in the PP + HBO + PP group than all other groups. Epithelialization and wound healing were better in the HBO and PP groups than in the control group. The greatest healing, epithelialization and vascularization was seen in the PP + HBO + PP group. The histopathological findings in the PP + HBO + PP group were better in each inner region than in the other groups.
Conclusion: Biochemical and histopathological parameters have shown that PP reduces ischemia and necrosis and increases oxygenation in flap healing by providing significant improvement thanks to the multiple molecular structures in its content.

1. Introduction

Random pattern skin flaps are frequently used for wound repair and reconstruction. Skin flaps or grafts have a wide application area in otolaryngology and plastic surgery. They are used after trauma, congenital diseases, and tumor excisions. In clinical practice, flap necrosis is a serious problem that may be caused by inadequate blood-nutritional support [1,2]. The most important goals in its treatment are reducing flap necrosis and increasing the viability of ischemic tissue.

There are several models showing that oxygen-induced free radicals lead to postischemic tissue injury [3]. Several studies have shown that superoxide radicals damage the microvascular network after ischemia [4,5]. In particular, the experimental island flap has been reported to undergo necrosis due to ischemia after reperfusion [6]. Vasoconstriction occurs upon activation of the sympathetic system following the blood flow interruption, causing necrosis in the distal part of the flap. Failure of microvascular anastomosis reperfusion includes ischemia, endothelial cell swelling, luminal occlusion, soft tissue damage caused by toxic free radicals, and their release during necrosis. Studies have shown that reactive oxygen radicals increase in postischemic injury [7]. The

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https://doi.org/10.1016/j.jtv.2021.02.008
Received 19 July 2020; Received in revised form 26 January 2021; Accepted 19 February 2021
Available online 26 February 2021

correlation between flap necrosis and oxygen-derived free radicals has shown that necrosis in experimental flap model can be reduced with different free radical absorbers, particularly antioxidants [8].

In the literature, there are studies reporting that hyperbaric oxygen (HBO) applications slow down the process of hypoxic ischemia and increase tissue healing. Oxidative mediators have been shown to decrease after HBO therapy and improve healing of the ischemic wounds than many medical treatments [9]. One of the most important indications for the use of HBO is to provide the viability of a flap with impaired blood supply [10].

Proxeed plus (PP) is a dietary supplement that provides sperm production, development, and maturation. It contains L-carnitine Fumarate (1.7 g), Acetyl-L-Carnitine (0.5 g), Selenium (50 mcg), Zinc (10 mg), Coenzyme Q10 (20 mg), Vitamin C (90 mg), Folic Acid (200 mcg), and Vitamin B12 (1.5 mcg). The effect of carnitine on the flap viability and ischemia has been shown in studies [11–13]. Selenium, which is a powerful antioxidant, has been shown to contribute to the viability of flap [14]. Studies have shown that zinc superoxide dismutase (SOD) is also effective in improving flap viability [15]. In a recent study, Coenzyme Q10 has been shown to improve the skin flap viability in rats when given through oral administration [16]. Vitamin C plays a key role in metabolism and has been shown to have significant contributions to the flap viability and prevention of ischemia [17–20]. Folic acid is one of the factors that play a role in cell maturation [21]. Vitamin B12 is also known to play a key role in cell maturation and DNA synthesis.

The aim of this study was to compare the efficacy of PP and HBO treatments in the prevention of ischemia and necrosis of local random McFarlane skin flap.

2. Material and method

2.1. Study design

Local ethics committee approval was obtained before starting the study. A total of 40 male Sprague–Dawley rats weighing 280–350 g were included. Rats were housed in an environment of 21 °C ± 1 with a background noise level of <50 dB, where they were given food and water freely, under the lighting cycles of 12 h of light and 12 h of dark. Animals were used in accordance with the National Regulation on the Care and Use of Laboratory Animals. The rats were divided into five groups, each containing eight rats.

Group 1 (n = 8) (Control): No application was performed except flap.
Group 2 (n = 8) (PP): Oral PP (150 mg/kg/day) was applied for two weeks after flap.
Group 3 (n = 8) (HBO): Ten sessions of HBO were applied after flap.
Group 4 (n = 8) (HBO + PP): Ten sessions of HBO and two weeks of oral PP (150 mg/kg/day) were applied after flap.
Group 5 (n = 8) (PP + HBO + PP): PP (150 mg/kg/day) was applied one week before the flap. Ten sessions of HBO and three weeks of oral PP (150 mg/kg/day) were applied after flap.

2.2. Surgical procedure

The dorsal part of each rat was shaved using a razor under anesthesia and cleansed with an antiseptic solution (Batticon). After ensuring the depth of anesthesia, a rectangular-shaped random flap with the 10 × 3 cm base in the caudal part of the animal was raised at the level of the panniculus carnosus [22]. Then, the flap was repositioned in its original position with 4–0 polydioxanone (PDS) sutures at 2 cm intervals (Fig. 1a and b). This procedure was routinely applied to all groups.

2.3. Drug administration

Proxeed Plus (Zinc-10 mg, Coenzyme Q10-20 mg, Vitamin C-90 mg, Fructose, L-carnitine fumarate-1.7 g, Acetyl-L-Carnitine, Folic Acid-200 mcg, Vitamin B12–1.5 mcg): Sigma-Tau Pharmaceuticals, Inc. Gaithersburg, Maryland.

Ketalar® 50 mg vial, Pfizer.

Rompun® 2% injection Solution, Bayer.

HBO therapy.

In the study, HBO therapy was applied to rats in Groups 3, 4 and 5. The HBO therapy was performed in the experimental HBO boiler at Istanbul University, Department of Underwater and Hyperbaric Medicine Department (Fig. 2). The boiler was equipped with a flowmeter and oxygen saturation devices that control oxygen input and output. The pressure in the boiler was increased to 2.5 ATA in 15 min, followed by 100% oxygen exposure at the same pressure for 60 min. This procedure was performed consecutively for 10 days.

2.4. Sample collection

In the study, anesthesia was applied to all groups when the procedure was finished. The status of the flap was photographed. Intracardiac

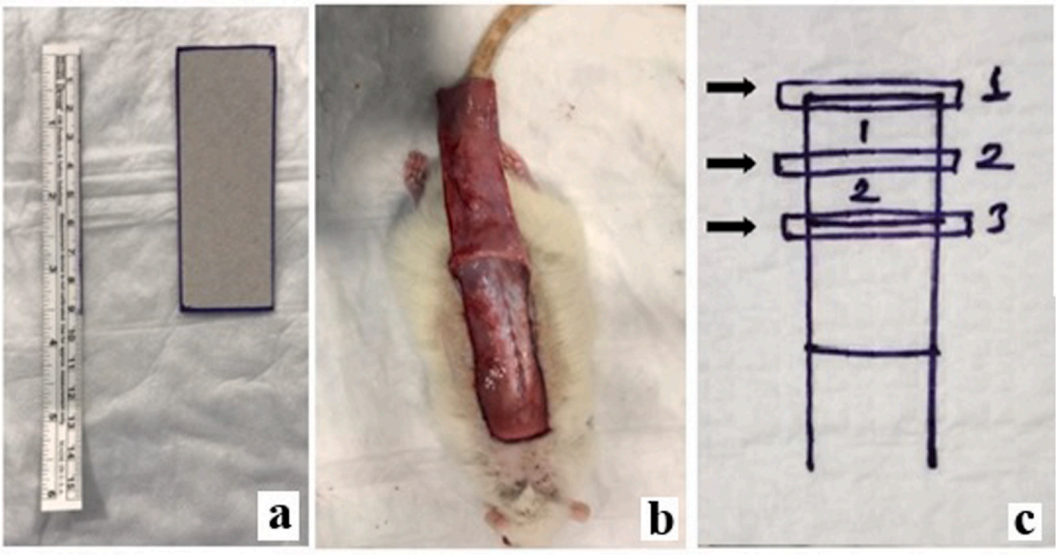

Fig. 1. Measurement of flap (a), surgical procedure (b), regions where samples were taken over the flap (c).

Fig. 2. Experimental HBO boiler.

blood samples were collected and centrifuged at 3000 rpm for 15 min for biochemical evaluation and then, plasma samples were separated. Oxidative stress parameters (total antioxidant status [TAS], total oxidant status [TOS], oxidative stress index [OSI]), inflammation parameters (interleukin-1 beta [IL-1β], IL-6, tumor necrosis factor-alpha [TNF-α]), and growth factors (transforming growth factor-beta [TGF-β], and vascular endothelial growth factor [VEGF]) were examined in biochemical evaluation.

Distal 1/3 of the flap was marked and samples were taken for biochemical and histopathological evaluation (Fig. 1c). For histopathological evaluation, flap samples were obtained from the first, second and third regions marked by the arrow on the distal 1/3 of the flap in the form of strips including the removed tissue. The samples were fixed with formalin and stored until the hematoxylin-eosin examination. In the biochemical evaluation, 0.5 × 0.5 cm samples were taken from the first and second regions in the midline and stored at −80°. Oxidative stress parameters (TAS, TOS, OSI), inflammation parameters (IL-1β, IL-6, TNF-α), and growth factors (TGF-β and VEGF) were examined.

2.5. Evaluation parameters

1. Evaluating the percentage of necrotic area: All flaps were photographed from a distance of 30 cm using a 12.1 Megapixel digital camera. The necrotic area was scanned and the necrotic percentage was determined through ImageJ software.
2. Biochemical Evaluation:
   - ➢ Oxidative Stress Parameters: TOS and TAS were detected in serum and tissue homogenates by using commercially available kits (Rel Assay, Turkey) with an autoanalyzer (Cobas Integra 800, Roche). TOS and TAS results were presented in mmol H2O2 equivalent/L and mmol Trolox equivalent/L, respectively [23,24]. The ratio of the TOS to the TAS revealed the OSI, which is used as an indicator for total oxidative stress [25].
   - ➢Inflammation Parameters (IL-1β, IL-6, TNF-α): Tissue samples of 5 mg from flap tissue and serum were transferred to Eppendorf tubes and 1 ml of water was added. Samples were, then, homogenized with ceramic balls. After centrifugation at 3000 rpm × 10 min at +4 °C, the supernatant was removed and protein determination was performed via the Bradford method. The inflammation parameters (IL-6, Rat IL-1β, and Rat TNF-α) were calculated spectrophotometrically using commercial enzyme-linked immunosorbent assay (ELISA) kits on a Thermo Scientific Varioskan multimode microplate reader.
   - ➢Growth Factor Parameters (TGF-β, VEGF): Tissue samples of 5 mg from flap tissue and serum were transferred to Eppendorf tubes and 1 ml of water was added. Samples were, then, homogenized with ceramic balls. After centrifugation at 3000 rpm × 10 min at +4 °C, the supernatant was removed and protein determination was performed via the Bradford method. The growth factor parameters used in the study (TGF-β and VEGF) were calculated photometrically using commercially available ELISA kits on a Thermo Scientific Varioskan multimode microplate reader. Results were divided into protein levels and expressed as per protein.
3. Histopathological evaluation

For light microscopic investigation, samples were placed in 10% formaldehyde, dehydrated in alcohol series (70%, 90%, 96% and 100%; respectively), cleared in xylene and embedded in paraffin. Sections (5 μm) were stained with hematoxylin and eosin (H&E) and examined with a video-light microscope (Nikon Eclipse i5, Tokyo, Japan; Nikon, DS-Fi1c).

2.6. Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) for Windows, Version 24.0 (IBM Corporation, Armonk, NY, USA). Mean, median values and standard distributions of all quantitative data were calculated. The Kolmogorov-Smirnov test was used to determine whether the distribution was normal. Kruskal–Wallis variance analysis was used to compare the biochemical and histological parameters of the groups. A p value of <0.05 was considered statistically significant. Mann–Whitney *U* test was used for intergroup comparison. Bonferroni correction was conducted. Tukey's HSD was used as a post hoc test.

3. Results

3.1. Evaluation of the percentage of the necrotic area

In our study, the necrosis percentage of the control group was found to be 54.87 ± 5.93%, which was significantly higher than all other groups ($p = 0.001$) (Fig. 3). There was no significant difference between the PP group (44.62 ± 5.60%) and HBO group (46. 50 ± 4.78%) in terms of necrosis percentage ($p = 0.942$) (Fig. 3). The necrosis percentage of the HBO + PP group (37.37 ± 3.73%) was significantly lower than both the PP group and the HBO group ($p = 0.045$ and $p = 0.007$, respectively) (Fig. 3). Although the necrosis percentage of the PP + HBO + PP group (30.87 ± 4.45%) was lower than those of the HBO + PP group, the difference was not statistically significant ($p = 0.089$) (Fig. 3).

3.2. Biochemical examination-plasma

3.2.1. Oxidative Stress Parameters

TOS: There was no significant difference between the control group and the HBO group in terms of TOS, whereas it was significantly lower in all other groups than the control group. The TOS value of the HBO + PP group was significantly lower than both the PP and HBO group. The TOS value of the PP + HBO + PP group was significantly lower than the HBO + PP group (Fig. 4).

TAS: The TAS value of the control group was significantly lower than all other groups. There was no significant difference between the PP and HBO groups in terms of TAS values. The TAS value of the HBO + PP group was significantly higher than the HBO group. The TAS value of the PP + HBO + PP group was significantly higher than both the PP and HBO + PP group. There was no significant difference between the PP + HBO + PP and HBO + PP group (Fig. 4).

OSI: The OSI value of the control group was higher than all other groups. There was no significant difference between the PP group and HBO group in terms of OSI values. The OSI value of the HBO + PP group was significantly lower than both the PP and HBO group. The OSI value of the PP + HBO + PP group was significantly lower than the HBO + PP group (Fig. 4).

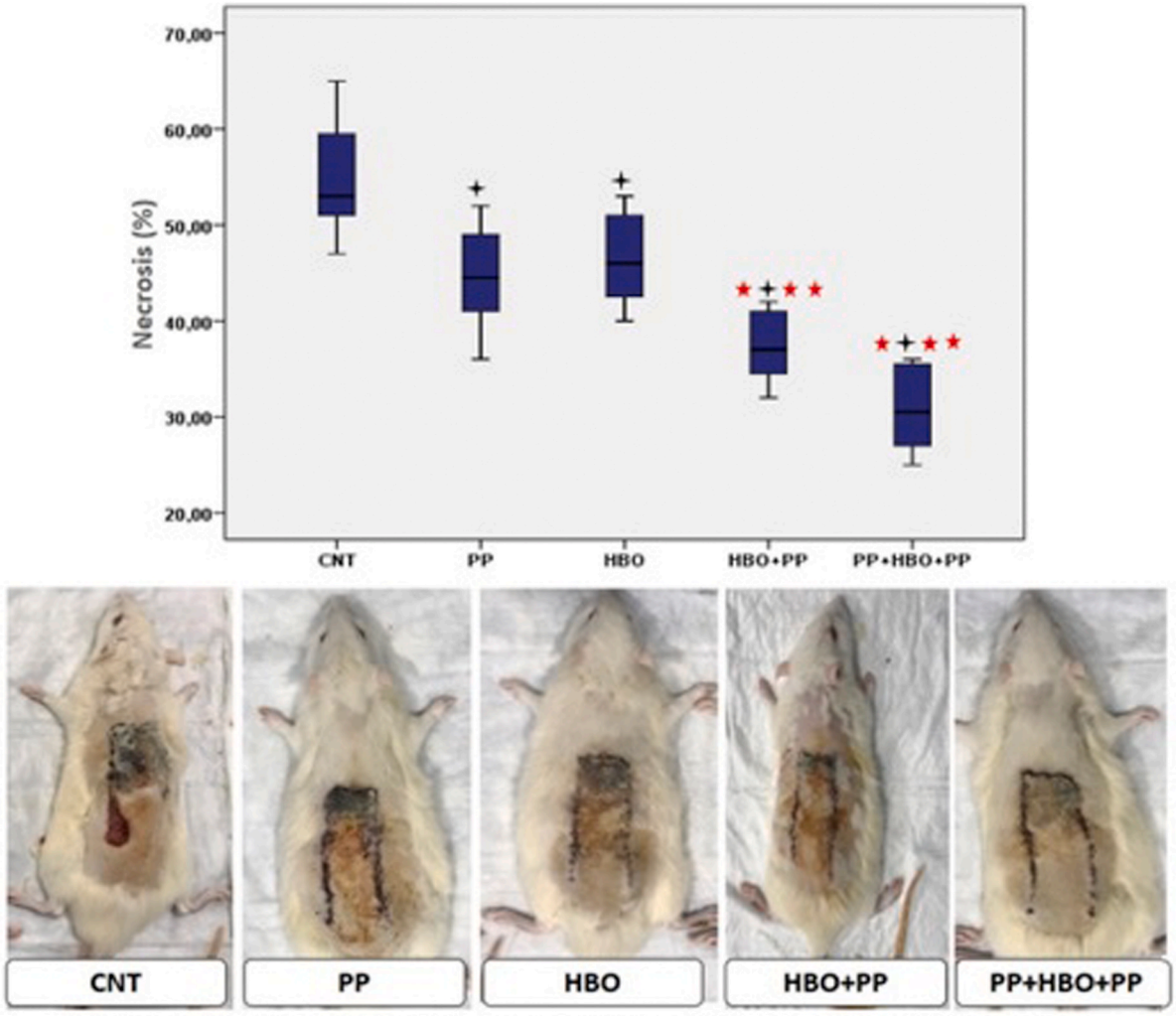

Fig. 3. a: Comparison of necrosis percentages of groups, Figure 3b:Representations of necrosis percentages of groups.

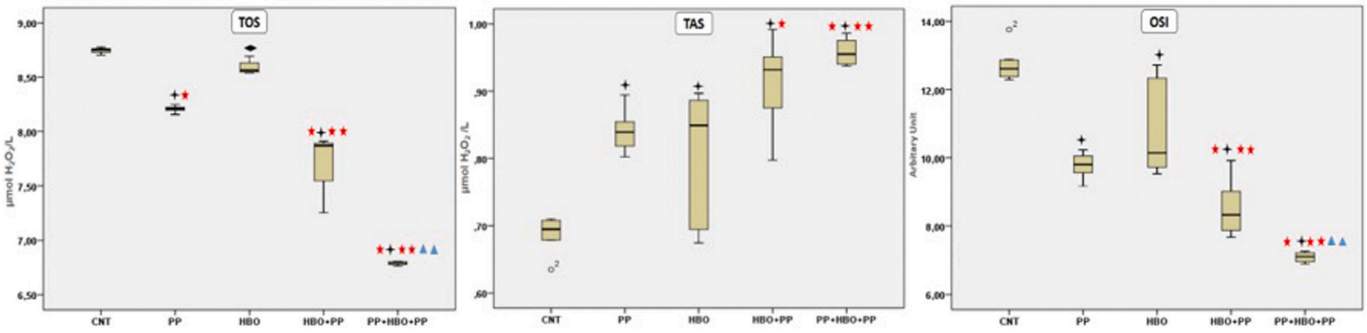

Fig. 4. Comparison of plasma oxidative stress parameters of groups.

3.2.2. Inflammation parameters

IL-1β: The values in the control group were significantly lower than PP, HBO + PP and PP + HBO + PP groups. Although IL-1β levels of the HBO group were lower than the control group, the difference between the groups was not significant. Although the IL-1β levels of the HBO + PP group were significantly lower than the PP group, there was no statistically significant difference between the HBO + PP group and the PP + HBO + PP group (Fig. 5).

IL-6: The values in the control group were significantly lower than PP, HBO + PP and PP + HBO + PP groups. Although IL-6 levels of the HBO group were lower than the control group, the difference between the groups was not significant. Although the IL-6 levels of the HBO + PP group were significantly lower than the PP group, there was no statistically significant difference between the HBO + PP group and the PP + HBO + PP group (Fig. 5).

TNF-α: The values in the control group were significantly lower than PP, HBO + PP and PP + HBO + PP groups. Although TNF-α levels of the HBO group were lower than the control group, the difference between the groups was not significant. The TNF-α value of the PP + HBO + PP group was lower than HBO + PP group but the difference was not

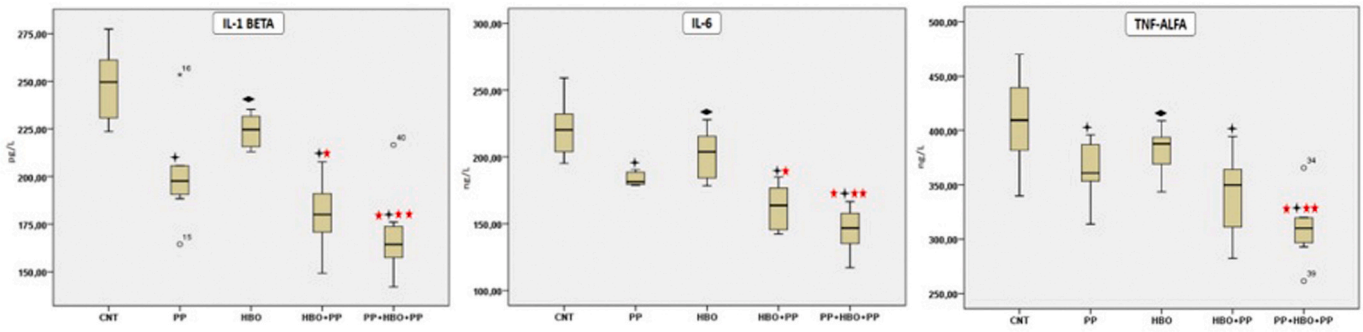

Fig. 5. Comparison of plasma inflammation parameters of groups.

statistically significant (Fig. 5).

3.2.3. Growth Factor Parameters

TGF-β: The TGF-β levels of the control group were significantly lower than PP, HBO + PP and PP + HBO + PP groups. Although TGF-β levels of the HBO group were lower than the control group, the difference between the groups was not significant. The TGF-β levels of the HBO + PP group were significantly higher than the HBO group. There was no significant difference between the PP + HBO + PP group and the HBO + PP group (Fig. 6).

VEGF: The VEGF levels of the control group were significantly lower than PP, HBO + PP and PP + HBO + PP groups. Although VEGF levels of the HBO group were higher than the control group, the difference between the groups was not significant. The VEGF value of the HBO + PP group was significantly higher than the HBO and PP group. The PP + HBO + PP group had significantly higher VEGF values than the HBO + PP group (Fig. 6).

3.3. Biochemical examination-tissue

3.3.1. Oxidative Stress Parameters

TOS: There was no significant difference between the control group and the HBO group and PP group in terms of TOS, whereas it was significantly lower in all other groups than the control group. There was no significant difference between the HBO + PP group and the PP and HBO groups in terms of TOS values. The TOS value of the PP + HBO + PP group was significantly lower than the HBO group (Fig. 7).

TAS: The TAS value of the control group was significantly lower than all other groups except the HBO group. There was no significant difference between the PP and HBO groups in terms of TAS values. The TAS value of the HBO + PP group was significantly higher than both PP and HBO groups. The TAS value of the PP + HBO + PP group was significantly higher than both the PP and HBO + PP group. There was no significant difference between the PP + HBO + PP and HBO + PP group (Fig. 7).

OSI: The OSI value of the control group was higher than all other groups. There was no significant difference between the PP group and HBO group in terms of OSI values. The OSI value of the HBO + PP group was significantly lower than the HBO group. Although the OSI value of PP + HBO + PP group was significantly lower than both PP and HBO group, there was no significant difference between the PP + HBO + PP and HBO + PP groups (Fig. 7).

3.3.2. Inflammation Parameters

IL-1β: The IL-1β values of the control group were significantly lower than all other groups. The IL-1β value of HBO group was significantly lower than the PP group. Although the IL-1β levels of the HBO + PP group were significantly lower than the PP group, there was no statistically significant difference between the HBO + PP and HBO groups. The IL-1β levels of the PP + HBO + PP group were significantly lower than all other groups (Fig. 8).

IL-6: The values in the control group were significantly lower than the PP, HBO + PP and PP + HBO + PP groups. Although IL-6 levels of the HBO group were lower than the control group, the difference between the groups was not significant. Although the IL-6 levels of the HBO + PP group were significantly lower than the HBO group, there was no statistically significant difference between the HBO + PP group and the PP + HBO + PP group (Fig. 8).

TNF-α: The TNF-α values of the control group were significantly lower than all other groups. The TNF-α values of the HBO group were significantly lower than the PP group. Although the TNF-α levels of the HBO + PP group were significantly lower than the PP group, there was no statistically significant difference between the HBO + PP and HBO groups. The TNF-α levels of the PP + HBO + PP group were significantly lower than all other groups (Fig. 8).

3.3.3. Growth Factor Parameters

TGF-β: The TGF-β levels of the control group were significantly lower than PP, HBO + PP and PP + HBO + PP groups. Although TGF-β levels of the HBO group were higher than the control group, the difference

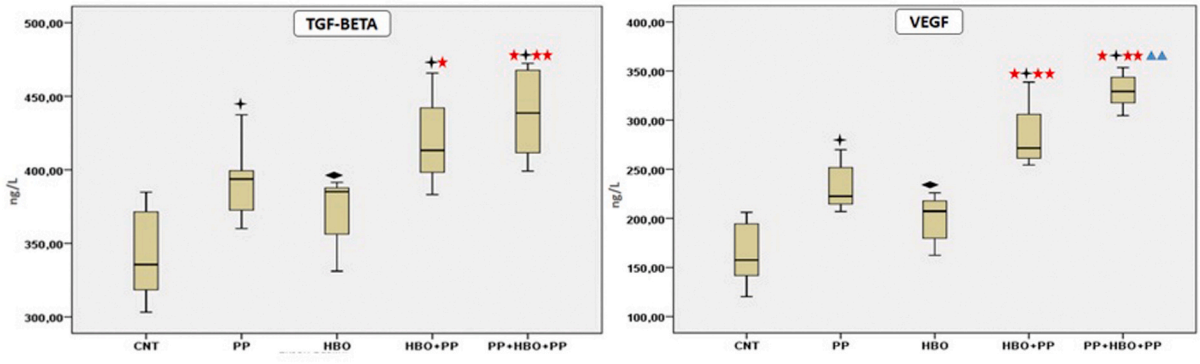

Fig. 6. Comparison of plasma growth factor parameters of groups.

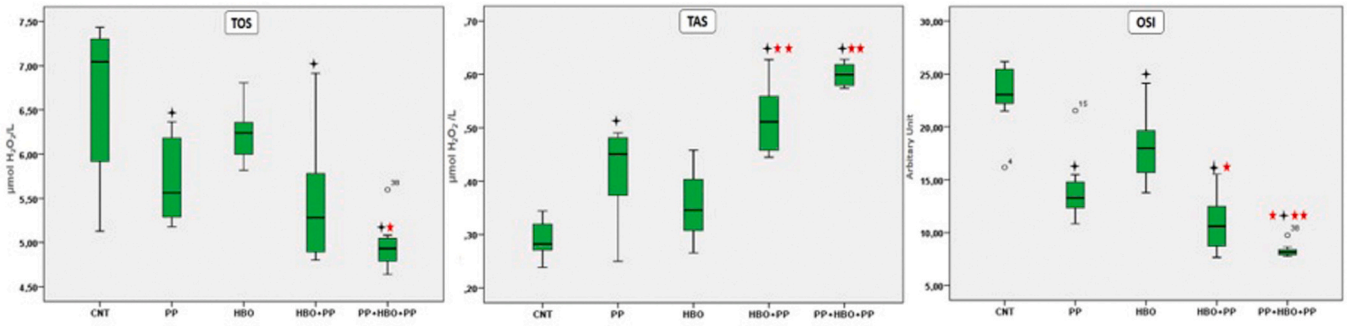

Fig. 7. Comparison of tissue oxidative stress parameters of groups.

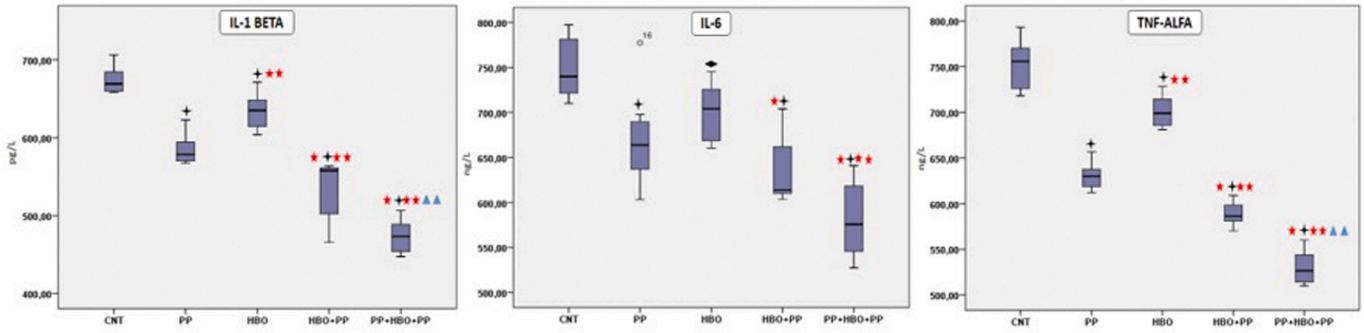

Fig. 8. Comparison of tissue inflammation parameters of groups.

between the groups was not significant. The TGF-β levels of the HBO + PP group were significantly higher than the HBO group. There was no significant difference between the PP + HBO + PP group and the HBO + PP group (Fig. 9).

VEGF: The VEGF value of the control group was lower than all other groups. The VEGF value of the PP group was significantly higher than the HBO group. The VEGF value of the HBO + PP group was significantly higher than the HBO and PP group. The PP + HBO + PP group had a significantly higher VEGF value than the HBO + PP group (Fig. 9).

3.4. Histopathological examination

In our study, the improvement was observed to decrease from the third to the first region in all groups. Evaluation by region, respectively;

3.4.1. First region

Grup 1 (CNT): The epithelium, hair follicles, sweat and sebaceous glands were absent in the wound area. A thick degenerated cellular and extracellular exfoliated material was observed at the surface (Fig. 10a).

Grup 2 (PP): There was severe degeneration in epidermis and dermis. Severe leukocyte infiltration was observed (Fig. 10b).

Grup 3(HBO): Severe degeneration was observed within the epidermis and dermis, hair follicles, sweat and sebaceous glands. A very thick exfoliated material was observed at the surface of the remaining narrowed dermis.A severe leukocyte infiltration and edema were observed. In some places epidermis and dermis was completely degenerated. Exfoliated material was just above the striated muscles of the back (Fig. 10c).

Grup 4 (HBO + PP): Severe epidermal, dermal degeneration, leukocyte infiltration and edema were observed. Hair follicles sweat and sebaceous glands were absent. There was a thick exfoliated material containing degenerated hair follicles and glands (Fig. 10d).

Grup 5 (PP + HBO + PP): the epithelium was somewhat degenerated, however still present. Hair follicles, sweat and sebaceous glands were observed. There was mild leukocyte infiltration. Exfoliated material was still present (Fig. 10e).

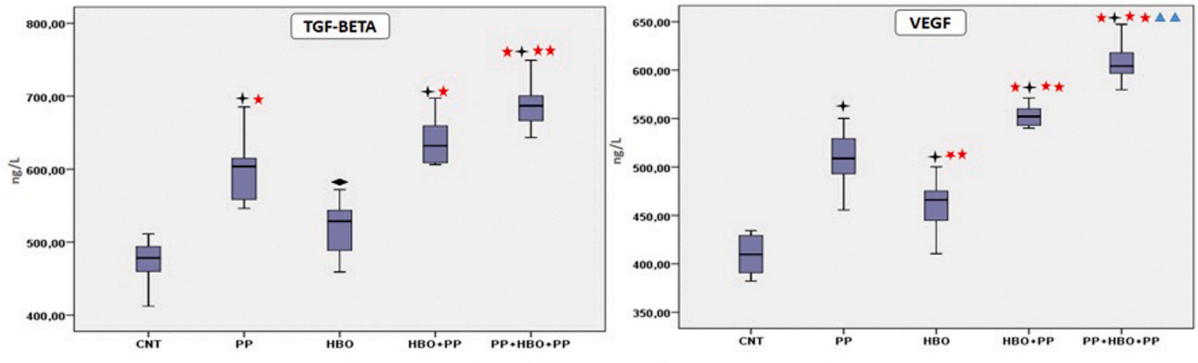

Fig. 9. Comparison of tissue growth factor parameters of groups.

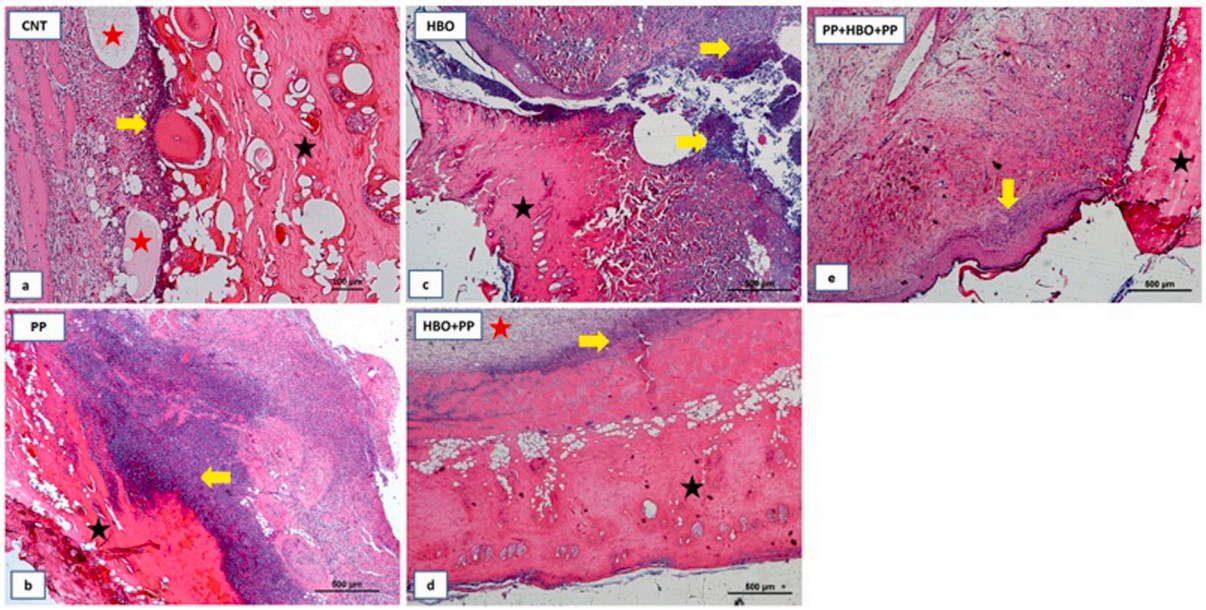

Fig. 10. Histopathological evaluation of the first region of the flaps.

3.4.2. Second region

Grup 1 (CNT): The epithelium, hair follicles, sweat and sebaceous glands were absent in the wound area. Degenerated hair follicles and glands were observed within the exfoliated material (Fig. 11a).

Grup 2 (PP): The epidermis and dermis were regenerated. Hair follicles and glands were not present. In some of the slides epidermis was degenerated. There were leukocyte infiltration and edema (Fig. 11b).

Grup 3 (HBO): Severe epidermal and dermal degeneration, leukocyte infiltration and edema were observed. Hair follicles and glands were not present (Fig. 11c).

Grup 4 (HBO + PP): The wound area was smaller than those of the other slides. The epidermis and dermis were regenerated. Hair follicles and glands were not present (Fig. 11d).

Grup 5 (PP + HBO + PP): In general epidermis and dermis were observed. Additionally some hair glands and sebaceous glands were still present. There was an exfoliated material at the apical surfaces even though it was thinner than those of the other groups (Fig. 11e).

3.4.3. Third region

Grup 1 (CNT): In a smaller area the epithelium was degenerated. Hair follicles, sweat and sebaceous glands were absent in the wound area (Fig. 12a).

Grup 2 (PP): Them both epidermis and dermis were successfully regenerated. Hair follicles and gland were observed (Fig. 12b).

Grup 3 (HBO): Generally epidermis and dermis were present. In a few area highly severe degeneration and leukocyte infiltration were observed. Additionally in those area a very thick exfoliated material was observed (Fig. 12c).

Grup 4 (HBO + PP): In generally epidermis and dermis were regenerated. Hair follicles and glands were present. In a few small areas,

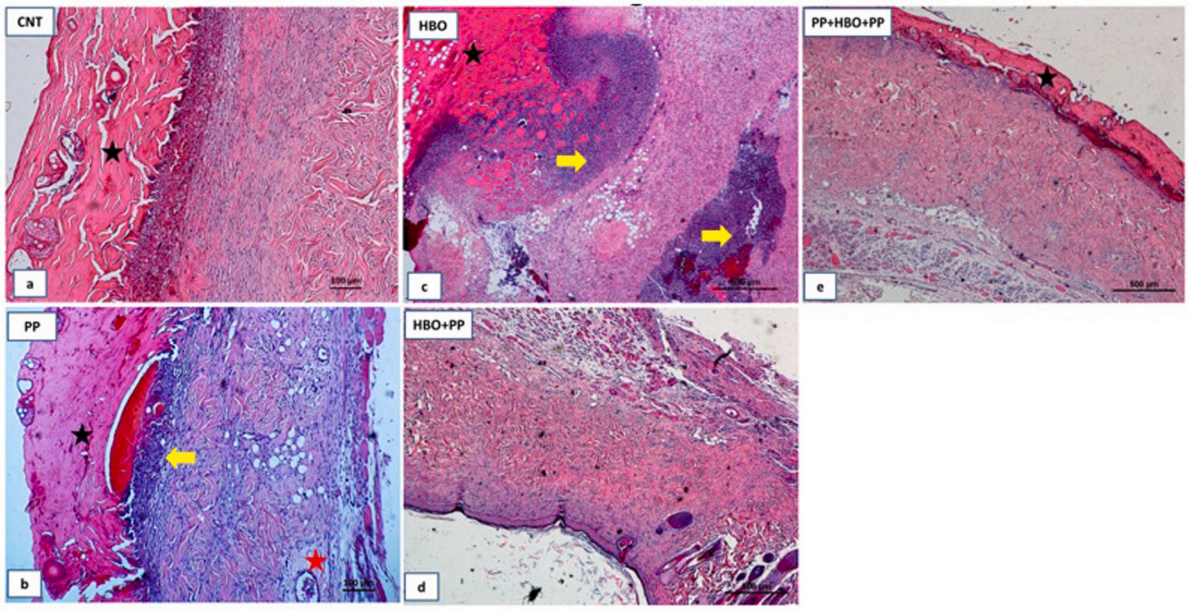

Fig. 11. Histopathological evaluation of the second region of the flaps.

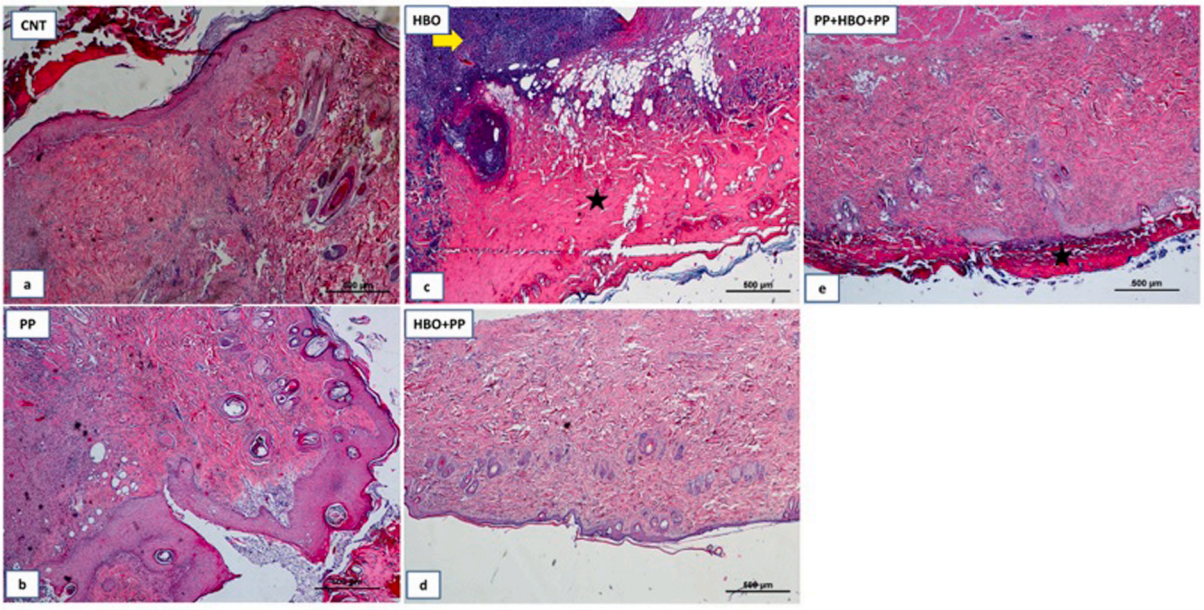

Fig. 12. Histopathological evaluation of the third region of the flap.

degeneration was still obvious (Fig. 12d).

Grup 5 (PP + HBO + PP): In generally epidermis and dermis were regenerated. Thin exfoliated material was present at the apical side. Hair follicles and gland were observed (Fig. 12e).

4. Discussion

The use of flaps in the restoration of cutaneous losses due to trauma, tumor resection, infection or ischemic necrosis is increasing in the practice of otolaryngology and plastic surgery. The use of flap has many benefits both functionally and cosmetically. However, the most important problem caused by flaps is possible partial or total ischemic problems. Flap viability may be deteriorated due to such reasons as the general condition of the patient, basal metabolic index, presence of systemic diseases and previous surgeries, leading to both functional and serious cosmetic problems.

Ischemia occurs in the last step of the chain of anatomical, hemodynamic and metabolic events that occurred after flap surgery. The pathophysiology of ischemia sheds light on some theories. In cases where flap circulation is deteriorated, intracellular metabolism tends to change from aerobic to anaerobic due to decreased tissue oxygen levels. This leads to the accumulation of lactate that will disrupt the membrane transport mechanism, followed by a decrease in the intracellular pH level and the depletion of adenosine triphosphate (ATP) stores in the ionic pumps. Free oxygen radicals are produced when the circulation returns to normal and oxygen re-enters the tissue. These substances are toxic to all cell structures containing proteins, carbohydrates, and fat. More importantly, endothelial damage will cause platelet and neutrophils to adhere to the vessel wall, which will initiate the coagulation cascade [26].

The flap distal is the region with the lowest ischemia tolerance. Cutaneous blood flow is the lowest in this region. Flow increases significantly 24 h after flap elevation. In skin flaps, this occurs by microvascular obstruction prior to tissue death (cellular edema, vasoconstriction of adrenergic origin), lumen obstruction (platelet plugs), aggregation and activation of leukocytes, or the combinations of all of them. Increased oxidative stress as a result of all these effects seriously threatens flap survival. All these steps should be considered in flap survival and substances developed and being developed must have the potential to affect one or more of these mechanisms.

In the literature, insufficient blood flow [27], inflammatory reactions [28] and oxidative stress [29] have been reported to be three important factors associated with flap necrosis. Several flap studies have been conducted to prevent these factors. One of the earliest studies on this subject has been conducted by Stewart et al. [29] and it has been shown that hyperbaric oxygen improves survival in random pattern skin flaps. Hyperbaric oxygenation was show to reduce the area of necrosis and preserved the morphology and collagen content in skin flaps of rats in a study by Rech et al. [30]. In the study of Oi et al., HBO was shown to increase flap viability in ischemia reperfusion rat model [31]. HBO demonstrates these effects by reducing inflammation and increasing perfusion [31]. Although HBO is still not used as the first treatment, it is an application that can be used as an aid in flap and wound healing. Because HBO therapy is not fully explained under wound healing in some places [32]. The use of HBO is still continuing, albeit partially, in cases with flap healing problems. The majority of cases with flap healing problems are smokers. In a study by Latifoglu et al. [33], calcium channel blockers were used to prevent necrosis in these patients and they were found to be effective. Furthermore, vitamin administration was performed to prevent ischemia-reperfusion in the flap. In the literature, vitamin C and high doses of vitamin E administration were evaluated in two different studies and they were found to be partially successful [33,34]. Superoxide dismutase has been used in studies aiming at improving survival by balancing and preventing oxidative damage in flaps [35]. Deferoxamine, an iron-binding and free radical scavenger, has also been shown to improve the survival of skin flaps [36]. Nakatsuka et al. [37] investigated the efficacy of glucocorticoids on capillary blood flow and flap viability in the skin and muscle-skin flaps in pigs.

There are many studies investigating the efficacy of different pharmacological agents in preventing and modifying ischemia in the skin flap. These agents include sympatholytics, vasodilators, prostaglandin inhibitors, anticoagulants and free radical scavengers [38]. Specific substances were also investigated. These include superoxide dismutase, catalase, allopurinol, Vitamin A, dexamethasone, cyclosporine A, methylprednisolone, azathioprine, heparin, intracellular adhesion molecule 1 monoclonal antibody (HOIST-1), cromolyn Sodium, vascular endothelial growth factor, lidocaine and topical prilocaine acid and nitric oxide, ginkgo biloba, aspirin, lidocaine and epinephrine, propofol, sildenafil, peptide-associated calcitonin gene, nicoboxil/nonivamide,

terazosin and propranolol, hydralazine, caffeic acid, and clopidogrel [22,39–52]. Although many agents have been studied, there is still no widely accepted and common molecule that has been routinely used. Therefore, in this study, we aimed to investigate the effect of PP (Zinc-10 mg, Coenzyme Q10-20 mg, Vitamin C-90 mg, Fructose, L-carnitine fumarate-1.7 g, Acetyl-L-Carnitine, Folic Acid-200 mcg, Vitamin B12–1.5 mcg), a multivitamin-multimineral supplement product, which has been used safely for a long time in routine clinical practice, on flap viability. It is a dietary supplement that provides sperm production, development, and maturation and has been used for about 30 years. Clinical trials have shown that ingredients contained in PP improve sperm motility, formation, maturation, number, and quality [53–57]. The interaction of PP with any drug is not known and there are no known side effects. However, less than 1% of patients have been reported to have mild gastrointestinal complaints.

Many of the substances contained in PP have been shown to be effective in preventing ischemia and necrosis in different studies. Zinc is involved in cytokine production and cell membrane stabilization and thus, prevents inflammatory lesions [58]. In the study by Schein et al. [59], zinc-containing Hr-CuZnSOD has been shown to have beneficial effects on flap viability. Coenzyme Q10 exhibits anti-inflammatory benefits by reducing the release of proinflammatory cytokines that occur during inflammatory damage [60]. In a recent study by Özalp et al. [61], coenzyme Q10 has been shown to help the improvement of flap viability in venous ischemia-reperfusion injury. Different studies have shown that coenzyme Q10 eliminates toxic effects through TNF-α inhibition [62,63]. In our study, the inflammation parameters (IL-1β, IL-6, and TNF-α) of the PP group were significantly lower than the control group. Inflammatory parameters of the PP + HBO + PP group were significantly lower than in other groups. In light of these findings, it is concluded that PP application is effective in preventing flap necrosis and its combined use with HBO increases its effectiveness. The TNF-α value of the PP + HBO + PP group was significantly lower than the HBO + PP group. This suggested that the effect would be greater when the PP application time was increased.

In a study by Kwon et al. [64], it has been reported that vitamin C administration has improved the flap viability and increased dosage is correlated with this situation. In a study by Georgopoulos et al. [65], vitamin C has been shown to have a less protective effect against ischemia-reperfusion injury compared to cimetidine and hydroxyzine. Bozkurt et al. [66] have reported in their experimental study that the antioxidant effect of vitamin C protects musculo-adipose-fasciocutaneous flaps against ischemia-reperfusion injury. In a recent study by Mokhimian et al. [67], it has been concluded that vitamin C used after testicular torsion prevents tissue damage and oxidative stress in the tunica vaginalis flap. In the present study, oxidative stress parameters decreased significantly in both tissue and plasma and antioxidant stress levels were significantly higher in PP treated groups compared to the control group.

L-carnitine is known to be a potent antioxidant and free radical scavenger [68]. In a study by Tellioglu et al. [69], L-Carnitine has been shown to provide a dose-dependent improvement in flap viability. In a study by Arslan et al. [70], L-carnitine has been reported to have a curative role in burns and limit necrosis, particularly in full-thickness burns. In the experimental study by Kargi et al. [71], L-carnitine and dexamethasone have been shown to have a synergistic effect in improving flap viability. In another experimental study by Aslan et al. [72], it has been concluded that the combined use of L-carnitine and vitamin C in the burned skin flap reduce necrosis. Ozkan et al. [73] have reported that L-carnitine administration has decreased the serum fibronectin level and improved flap viability. In a study by Scioli et al. [74], L-carnitine has been found to eliminate microvascular endothelial dysfunction and to improve the skin flap viability and to enhance wound healing and vascular angiogenesis in rats. In this study, L-carnitine's role of increasing VEGF levels has been reported to be effective in increasing vascular angiogenesis [74]. In our study, the growth factor parameters (TGF-β and VEGF) were found to be significantly higher in PP treated groups than in the control group. We believe that this is due to the content of L-carnitine. Particularly the finding that the healing was better in the PP + HBO + PP group supports the literature.

Folic acid regulates many different pathways such as cell growth, differentiation, DNA repair, apoptosis and carcinogenesis [75]. Vitamin B12 has serious effects on cellular redox balance and oxidative stress [76]. Furthermore, it reduces the transcription factor nuclear factor kappa B (NF-κB) levels, inhibits nitric oxide synthesis and promotes oxidative phosphorylation [77]. In the present study, OSI levels were significantly lower in all groups in both tissue and plasma than in the control group.

Selenium has a significant role in antioxidant defense systems and shows its most important effect through the active region of GPx selenoenzyme. This enzyme not only protects cells against free radicals but also promotes the repair of membrane lipid molecules via the realkylation mechanism [78]. Single-dose administration of selenium nanoparticles has been shown to inhibit free oxygen radicals in cell culture induced by cisplatin and to block apoptosis [79]. In a recent experimental study by Tenekeci et al. [80], selenium administration has been shown to prevent ischemia-reperfusion injury in tissue. Selenium exhibits this preventive effect by reducing oxidative stress, increasing vascular density, enhancing neutrophil infiltration, and suppressing inflammation [81].

According to the International Submarine and Hyperbaric Medicine Association, HBO therapy is applied rather than normal graft or flap applications, or it is recommended to use perfusion and hypoxia for other reasons [82]. HBO treatment increases the oxygen carrying capacity of plasma by 7% [82]. A different mechanism affects flap healing. The molecules in this message increase flap healing and viability through different mechanisms. Selenium has an antioxidant effect, Zinc and Coenzyme Q increase flap viability, Vitamin C prevents ischemia, increases folic acid cell maturation, and vitamin B12 acts with its role in DNA synthesis (14–21). Thus, PP flap healing is accelerated in different steps that may not be affected by HBO. In the present study, epidermis and dermis regeneration were found to be better in the PP group than in the control group. The histopathologic findings of the HBO + PP group were better than the control group but worse than the PP + HBO + PP. Epithelial and dermis regeneration was found to be better particularly in the PP + HBO + PP group compared to other groups. When the groups were evaluated in terms of tissue samples taken from the second and third regions, the healing status of hair follicles and sebaceous glands was better in the PP + HBO + PP group compared to the other groups. These data show that PP improves histopathological parameters. Based on these parameters, when the groups were compared in terms of flap necrosis, the highest necrosis percentage was seen to be in the control group. The necrosis percentage was significantly lower in all other groups compared to the control group. There was no significant difference between the PP and HBO groups in terms of necrosis percentage. The necrosis percentage of the HBO + PP group was significantly lower than that of both HBO and PP groups. Although the necrosis percentage of the PP + HBO + PP group was lower than those of the HBO + PP group, the difference was not statistically significant. In light of these data, we concluded that PP is as effective as HBO therapy in the prevention of necrosis, its effect is increased when combined with HBO, and there is a further increase in its effect when applied before and after surgery.

4.1. Strengths of the study

1. Proxeed plus is a safe combination with long-term clinical use.
2. There are many studies in the literature showing that the molecules contained in PP contribute individually to improve flap viability and in the present study, these molecules have been used in combination.
3. In the study, oxidative stress, inflammation and growth factor parameters were evaluated.

4. Biochemical evaluation was performed on both plasma and tissue.
5. Histopathological evaluation was performed.
6. The applications of PP were grouped into three: alone, combined used with HBO, and application before and after surgery.
7. The study included HBO therapy which is the most commonly used treatment modality to improve flap viability in clinical practice.

The weakness of our study was the lack of immunohistochemical evaluation.

5. Conclusion

This is the first study evaluating the efficacy of PP that contains combined support molecules in preventing ischemia and necrosis in the rat skin flap model. Proxeed Plus has been shown to prevent tissue ischemia-reperfusion injury and tissue necrosis with the biochemical and histopathological data. We concluded that PP has similar effects with HBO, its effect increases when combined with HBO, and it is more effective when used both before and after the surgery.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Matsumura H, Yoshizawa N, Vedder NB, Watanabe K. Preconditioning of the distal portion of a rat random pattern skin flap. Br J Plast Surg 2001;54:58–61.
[2] Richards L, Lineaweaver WC, Stile F, Zhang J, Zhang F. Effect of hyperbaric oxygen therapy on the tubed pedicle flap survival in a rat model. Ann Plast Surg 2003;50:51–6.
[3] Mccord JM. Oxygen-derived free radicals in postischemic tissue injury. N. Engi.]. Me 1985;312:159–63.
[4] Korthuis RJ, Grander DN, Townsley MI, et al. The role of oxygen-derived free radicals in ischemia-induced increases in canine skeletal muscle vascular permeability. Circ Res 1985;57:599–609.
[5] Granger DN, Rutil RG, Mccord JM. Superoxide radicals in feline intestinal ischemia. Gastroenterology 1981;81:22–9.
[6] Del Maestro R. An approach to free radicals in medicine and biology. Acta Physiol Scand Suppl 1980;492:153–68.
[7] Manson PN, Anthenelli RM, Im MJ, et al. The role of oxygenfree radicals in ischemic tissue injury in island skin flaps. Ann Surg 1983;198:87–90.
[8] Im MJ, Manson PN, Bulkley GB, et al. Effects of superoxide dismutase and allopurinol on the survival of acute island skin flaps. Ann Surg 1985;201:357–9.
[9] Sasaki GH, Pang CY. Hemodynamics and viability of acute neurovascular island skin flaps in rats. Plast Reconstr Surg 1980;65:152–9.
[10] Zhang Q, Chang Q, Cox RA, Gong X, Gould LJ. Hyperbaric oxygen attenuatesapoptosis and decreases inflammation in an ischemic wound model. J Invest Dermatol 2008;128(8):2102–12.
[11] McCrary BF. Hyperbaric oxygen (HBO2) treatment for a failing facial flap. Postgrad Med 2007;83:975.
[12] Arslan E1, Milcan A, Unal S, Demirkan F, Polat A, Bagdatoglu O, Aksoy A, Polat G. The effects of carnitine on distally-burned dorsal skin flap: an experimental study in rats. Burns 2003;29(3):221–7.
[13] Kargi E, Deren O, Babuccu O, Hosnuter M, Erdogan B. Dual synergistic effect: the effect of dexamethasone plus carnitine on skin flap survival. Ann Plast Surg 2004;53(5):488–91.
[14] Tellioğlu AT, Uras KA, Yilmaz T, Alagözlü H, Tekdemir I, Karabağ O. The effect of carnitine on random-pattern flap survival in rats. Plast Reconstr Surg 2001;108(4):959–62.
[15] Tenekeci G, Bilen BT, Turkoz Y, Sahin N, Bulam N, Erdemli ME. The effect of selenium on ischemia-reperfusion injury: an experimental study on a transverse rectus abdominis musculocutaneous flap model. J Craniofac Surg 2016;27(1):242–6.
[16] Lewin A, Lavon H. Mol Aspect Med 1997;18:213–9.
[17] Can A, Temel M, Dokuyucu R, Mutaf M. The effect of coenzyme Q10 (ubiquinone) on random pattern skin flap survival in rat model. Ann Plast Surg 2015;77(2):9–14.
[18] Kwon ST, Jeong JH, Eun SC, Baek RM, Minn KW, Heo CY. The effects of mega-dose ascorbic acid on skin flap survival: experimental study on rats. J Plast Reconstr Aesthetic Surg 2009;62(12):641–2.
[19] Tomur A, Etlik O, Gundogan NU. Hyperbaric oxygenation and antioxidant vitamin combination reduces ischemia-reperfusion injury in arat epigastric island skin-flap model. J Basic Clin Physiol Pharmacol 2005;16(4):275–85.
[20] Arslan E, Basterzi Y, Aksoy A, Majka C, Unal S, Sari A, Demirkan F. The additive effects of carnitine and ascorbic acid on distally burned dorsal skin flap in rats. Med Sci Mon Int Med J Exp Clin Res 2005;11(6):176–80.
[21] Wong WY, Merkus HM, Thomas CM, et al. Fertil Steril 2002;77(3):491–8.
[22] McFarlane RM, DeYoung G, Henry RA. The design of pedicle flap in the rat to study necrosis and its prevention. Plast Reconstr Surg 1965;35(2):177–82.
[23] Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem 2014;47:326–32.
[24] Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 2005;38(12):1103–11.
[25] Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 2004;37(4):277–85.
[26] Harma M, Kocyigit A, Erel O. Increased DNA damage in patients with complete hydatidiform mole. Swiss Med Wkly 2003;583(1):49–54.
[27] Russel RC, Roth AC, Kucan JO, Zook EG. Reperfusion injury and oxygen free radicals. A review J. Plas. Recon. Surg. 1989;5(1):79–84.
[28] Yang M, Sheng L, Li H, Weng R, Li QF. Improvement of the skin flap survival with the bone marrow-derived mononuclear cells transplantation in a rat model. Microsurgery 2010;30:275–81.
[29] Kim HJ, et al. Anti-inflammatory effects of anthocyanins from black soybean seed coat on the keratinocytes and ischemiareperfusion injury in rat skin flaps. Microsurgery 2012;32:563–70.
[30] Rech FV, Fagundes AL, Simões RS, Florencio-Silva R, Sasso GR, Taha MO, Fagundes DJ. Action of hyperbaric oxygenation in the rat skin flap. Acta Cir Bras 2015 Apr;30(4):235–41.
[31] Qi Z, Gao CJ, Wang YB, Ma XM, Zhao L, Liu FJ, Liu XH, Sun XJ, Wang XJ. Effects of hyperbaric oxygen preconditioning on ischemia-reperfusion inflammation and skin flap survival. Chin Med J (Engl). 2013 Oct;126(20):3904–9.
[32] Harl MJ. Defining the role of hyperbaric oxygen therapy as an adjunct to reconstructive surgery. Surg Clin 2020 Aug;100(4):777–85.
[33] Latifoğlu O, Atabay K, Çelebi C, Çenetoğlu S, Baran NK. Nikotin etkisi altındaki deri fleplerinin yaşayan uzunluğuna nifedipinin etkisinin araştırılması. Türk Plastik Cerrahi Dergisi 1995;3(3):144–51.
[34] Zaccaria A, Weinzweig N, Yoshitake M, Matsuda T, Cohen M. Vitamin C reduces ischemia – reperfusion injury in rat epigastric island skin flap model. Ann Plast Surg 1994;33:620–3.
[35] Axfort – Gately RA, Wilson GJ. Myocardial infarct size reduction by single high dose repeated log dose vitamin E supplemantation in rabbits. Can JCardiol 1993;9:94–8.
[36] Manson PN, Anthenelli RM, İm MJ. The role of oxygen radicals in ischemic tissue injury in island skin flaps. Ann Surg 1983;198:87–90.
[37] Karacaoglan N, Akbas H. Effect of parenteral pentoxiphylline and topical nitroglycerine on skin flap survival. Otolaryngol Head Neck Surg 1999;120(2):272.
[38] Nakatsuka T, Pang CY, Neligan P. Effect of glucocorticoid treatment on skin capillary blood flow and viability in cutaneous and myocutaneous flaps in the pig. Plast Reconstr Surg 1985;76:374.
[39] Huemer GM, Wechselberger G, Otto-Schoeller A, Gurunluoglu R, Piza-Katzer H, Schoeller T. Improved dorsal randompattern skin flap survival in rats with a topically applied combination of Nonivamide and Nicoboxil. Plast Reconstr Surg 2003;111(3):1207–11.
[40] Hart K, Baur D, Hodam J, Lesoon-Wood L, Parham M, Keith K, Vasquez R, Ager E, Pizarro J. Short- and long-term effects of sildenafil on skin flap survival in rats. Laryngoscope 2006;116:522–8.
[41] Huemer GM, Wechselberger G, Otto-Schoeller A, Gurunluoglu R, Piza-Katzer H, Schoeller T. Improved dorsal randompattern skin flap survival in rats with a topically applied combination of Nonivamide and Nicoboxil. Plast Reconstr Surg 2003;111(3):1207–11.
[42] Bittencourt RC, Biondo-Simões ML, Paula JB, Martynetz J, Groth A. Influence of minoxidil on ischemic cutaneous flaps in rats. Acta Cir Bras 2005;20(6):450–4.
[43] Komorowska-Timek E, Timek TA, Brevetti LS, Szuba A, Stuelinger M, Hardesty RA, Buncke HJ. Oral administration of L-arginine decreases necrosis of the epigastric skin flap in the rat. Ann Plast Surg 2004;53(1):73–8.
[44] Souza Filho MV, Leal PR, Sbalchiero JC, Marques MJ. Effect of ginkgo biloba extract on skin flap survival in rats. Rev Soc Bras Cir Plast 2002;17(2):45–56.
[45] Ghavami A, Nutt MP, Hardy SP. Heat shock protein and high-dose aspirin: effects on random skin flap survival in a rat model. Ann Plast Surg 2002;48(1):60–7.
[46] Tyner TR, Shahbazian R, Nakashima J, Kane S, Sian K, Yamaguchi KT. Propofol improves skin flap survival in a rat model: correlating reduction in flap-induced neutrophil activity. Ann Plast Surg 2004;53(3):273–7.
[47] Sarifakioglu N, Gokrem S, Ates L, Akbuga UB, Aslan G. The influence of sildenafil on random skin flap survival in rats: an experimental study. Br J Plast Surg 2004;57(8):769–72.
[48] Esteves Junior I, Ferreira LM, Liebano RE. Calcitonin gene-related peptide by iontophoresis on the viability of the randon skin flaps in rats. Acta Cir Bras 2004;19(6):626–9.
[49] Fonseca AV, Ferreira LM, Gomes HC, Percario S. Terazosin and propanolol as blockers to the deleterious effect of nicotine in a random skin flap in the rat. Acta Cir Bras 2004;19(3):186–95.
[50] Yoshida WB, Campos EB. Ischemia and reperfusion in skin flaps: effects of mannitol and vitamin C in reducing necrosis area in a rat experimental model. Acta Cir Bras 2005;20(5):358–63.
[51] Junior IE, Masson IB, Ferreira LM, Liebano RE, Baldan C, Gomes AC. Topical administration of hydralazine hydrochloride on the viability of randon skin flaps in rats. Acta Cir Bras 2005;20(2):164–7.

[52] Aydogan H, Gurlek A, Parlakpinar H, Askar I, Bay-Karabulut A, Aydogan N, Fariz A, Acet A. Beneficial effects of caffeic acid phenethyl ester (CAPE) on the ischaemia-reperfusion injury in rat skin flaps. J Plast Reconstr Aesthetic Surg 2007; 60(5):563–8.
[53] Ersoy A, Sevin K, Sevin A, Serel S. Effects of clopidogrel on survival of rat skin flaps. J Plast Reconstr Aesthetic Surg 2007;60(8):861–3.
[54] Agarwal A, Said TM. Reprod Biomed Online 2004;8(4):376–84.
[55] Saleh RA, Agarwal A, Nada EA, et al. Fertil Steril 2003;79:1597–605.
[56] Data on file. Proxeed® plus product information Sigma-Tau Pharmaceuticals, Inc.
[57] Hendin BN, Kolettis PN, Sharma RK, et al. J Urol 1999;161(6):1831–4.
[58] Kolettis PN, Sharma RK, Pasqualotto FF, et al. Fertil Steril 1999;71(2):249–55.
[59] Kaur K, Gupta R, Saraf SA, Saraf SK. Zinc: the metal of life. Compr Rev Food Sci Food Saf 2014;13:358–75.
[60] Schein O, Westreich M, Shalom A. Effect of intradermal human recombinant copper-zinc superoxide dismutase on random pattern flaps in rats. Head Neck 2013;35(9):1265–8.
[61] Schmelzer C, Lorenz G, Lindner I, Rimbach G, Niklowitz P, Menke T, Döring F. Effects of Coenzyme Q10 on TNF-alpha secretion in human and murine monocytic cell lines. Biofactors 2007;31:35–41.
[62] Özalp B, Elbey H, Aydın H, Tekkesin MS, Uzun H. The effect of coenzyme Q10 on venous ischemia reperfusion injury. J Surg Res 2016;204(2):304–10.
[63] Takaya T, Kawashima S, Shinohara M, Yamashita T, Toh R, Sasaki N, Inoue N, Hirata K, Yokoyama M. Angiotensin II type 1 receptor blocker telmisartan suppresses superoxide production and reduces atherosclerotic lesion formation in apolipoprotein E-deficient mice. Atherosclerosis 2006;186:402–10.
[64] Morishima M, Wang Y, Akiyoshi Y, Miyamoto S, Ono K. Telmisartan, an angiotensin II type 1 receptor antagonist, attenuates T-type Ca2+ channel expression in neonatal rat cardiomyocytes. Eur J Pharmacol 2009;609:105–12.
[65] Kwon ST, Jeong JH, Eun SC, Baek RM, Minn KW, Heo CY, Kwon ST, Jeong JH, Eun SC, Baek RM, Minn KW, Heo CY. J Plast Reconstr Aesthetic Surg 2009;62(12): 641–2.
[66] Georgopoulos S, Mastorakos D, Kondi-Pafiti A, Katsenis K, Arkadopoulos N, Kannas D, Archontaki M, Vestarchis N, Kokkalis G. Hydroxyzine, cimetidine and vitamin C in reducing skin flap necrosis in ischemia-reperfusion injury in rats. A comparative study. J BUON 2012;17(2):377–82.
[67] Bozkurt M, Kapi E, Kulahci Y, Gedik E, Ozekinci S, Isik FB, Celik Y, Selcuk CT. Kuvat SVAntioxidant support in composite musculo-adipose-fasciocutaneous flap applications: an experimental study. J Plast Surg Hand Surg 2014;48(1):44–50.
[68] Moghimian M, Soltani M, Abtahi H, Shokoohi M. Effect of vitamin C on tissue damage and oxidative stress following tunica vaginalis flapcoverage after testicular torsion. J Pediatr Surg 2017;52(10):1651–5.
[69] Arockia Rani PJ, Panneerselvam C. Carnitine as a free radical scavenger in aging. Exp Gerontol 2001;36:1713–26.
[70] Tellioğlu AT, Uras KA, Yilmaz T, Alagözlü H, Tekdemir I, Karabağ O. The effect of carnitine on random-pattern flap survival in rats. Plast Reconstr Surg 2001;108(4): 959–62.
[71] Arslan E, Milcan A, Unal S, Demirkan F, Polat A, Bagdatoglu O, Aksoy A, Polat G. The effects of carnitine on distally-burned dorsal skin flap: an experimental study in rats. Burns 2003;29(3):221–7.
[72] Kargi E, Deren O, Babuccu O, Hosnuter M, Erdogan B. Dual synergistic effect: the effect of dexamethasone plus carnitine on skin flap survival. Ann Plast Surg 2004; 53(5):488–91.
[73] Arslan E, Basterzi Y, Aksoy A, Majka C, Unal S, Sari A, Demirkan F. The additive effects of carnitine and ascorbic acid on distally burned dorsal skin flap in rats. Med Sci Mon Int Med J Exp Clin Res 2005;11(6):176–80.
[74] Ozkan OF, Komurcu E, Arik MK, Kemik AS, Tas S, Nusran G. Relationship between serum fibronectin levels and carnitine administration: an experimental study in rats. Int Wound J 2014;11(6):718–22.
[75] Scioli MG, Lo Giudice P, Bielli A, Tarallo V, De Rosa A, De Falco S, Orlandi A. Propionyl-L-carnitine enhances wound healing and counteracts microvascular endothelial cell dysfunction. PloS One 2015;16(10):0140697. 10.
[76] Trujillo E, Davis C, Milner J. Nutrigenomics, proteomics, metabolomics, and the practice of dietetics. J Am Diet Assoc 2006;106:403–13.
[77] Jessica AS, Nynke EH, Henk JB, et al. S-Adenosylhomocysteine induces apoptosis and phosphatidylserine exposure in endothelial cells independent of homocysteine. Atherosclerosis 2012;221:48–54.
[78] Bito T, Misaki T, Yabuta Y, Ishikawa T, Kawano T, Watanabe F. Vitamin B12 deficiency results n severe oxidative stress, leading to memory retention impairment in Caenorhabditis elegans Redox. Biol 2017;11:21–9.
[79] Soudani N, Troudi A, Bouaziz H, Ben Amara I, Boudawara T, Zeghal N. Cardioprotective effects of selenium on chromium (VI)-induced toxicity in female rats. Ecotoxicol Environ Saf 2011;74:513–20.
[80] Li Y, Li X, Wong YS, et al. The reversal of cisplatin-induced nephrotoxicity by selenium nanoparticles functionalized with 11-mercapto-1-undecanol by inhibition of ROS-mediated apoptosis. Biomaterials 2011;32:9068–76.
[81] Tenekeci G, Bilen BT, Turkoz Y, Sahin N, Bulam N, Erdemli ME. The effect of selenium on ischemia-reperfusion injury: an experimental study on a transverse rectus abdominis musculocutaneous flap model. J Craniofac Surg 2016;27(1): 242–6.
[82] Jones MW, Cooper JS. Hyperbaric therapy for skin grafts and flaps. In: StatPearls [internet]. Treasure island (FL): StatPearls publishing; 2020 jan; 2020 Oct 1.