ABSTRACT
The objective of this study was to evaluate the histopathologic effects of hyperbaric oxygen (HBO) therapy on the rat nasal mucosa. Twelve adult Sprague-Dawley rats, each weighing 180–220 g, were used as experimental animals. The rats were divided into HBO (hyperbaric oxygen) and control group. The rats in the HBO group (n = 6) were placed into a 20-liter HBO chamber (2.5 atmospheres absolute [ATA], 25–26 °C with 100 % oxygen) for 90 min per day. The rats received hyperbaric oxygen over a period of 7 days. The rats in the control group (n = 6) were not given HBO. All animals were sacrificed at the end of the study, and nasal tissue samples were prepared. The sections were stained with Haematoxylin and Eosin (H-E), Periodic acid-Schiff (PAS) and Trichrome-Masson to observe the under a light microscope. Immunoreactivity of pseudostratified epithelial cells of the nasal mucosa was assessed with E-cadherin expression by immunohistochemical staining. There were significant differences in the average histopathological score between the groups exposed and non-exposed to HBO. In the HBO group, degenerative changes in epithelial cells were observed. The goblet cells showed expansion of their structure. Mononuclear lymphocyte infiltration, dilation of blood vessels, and hemorrhage were observed in considerable areas of connective tissue. In the immunohistochemical evaluation of E-cadherin expression, there were some significant differences between the two groups.

Keywords: hyperbaric oxygen, rat, nasal mucosa, E-cadherin, immunohistochemistry

Introduction
Hyperbaric oxygen (HBO) therapy is defined as breathing 100 % oxygen under a pressure greater than 1 atmosphere oxygen in a pressure chamber. This therapy was introduced by Gray et al. (3) in 1953 and is increasingly used in a number of areas of medical practice to treat patients with numerous disorders. The Undersea and Hyperbaric Medical Society has approved the use of HBO in the treatment of the following situations: air or gas embolism, carbon monoxide poisoning, gas gangrene, crush injury, decompression sickness, arterial insufficiencies such as central retinal artery occlusion, severe anemia, intracranial abscess, necrotizing soft tissue infections, refractory osteomyelitis, delayed radiation injury, compromised grafts and flaps, acute thermal burn injury and idiopathic sudden sensorineural hearing loss (2, 7, 9). On the other hand, as with all medical procedures and treatments, some potential adverse effects may result from exposure to HBO. The knowledge has been developed from the findings of thousands of published animal and human studies defining the adverse effects of the HBO therapy. The main categories of these potential adverse effects are barotraumatic lesions (middle ear, nasal sinuses, inner ear, lung and teeth), oxygen toxicity (central nervous system and lung), ocular effects (myopia and cataract) and anxiety (6).

HBO is administered with inhalation, and thus there might be some adverse effects on nasal mucosal surface. To the best of our knowledge, there are only a few reports about the effects of HBO on this area. The aim of this study was to evaluate the histopathologic effects of HBO on the rat nasal mucosa.

Materials and Methods
Animals and application of HBO
The study protocol was approved by the Animal Research Committee of Dicle University (DUHADEK), Turkey. Twelve adult Sprague-Dawley rats, each weighing 180–220 g, were used as experimental animals. The animals were group-housed (6 per cage) under standard conditions (21 ± 2 °C) in the Animal Health and Research Center of Dicle University (DUSAM). The animals were fed ad libitum with water and standard laboratory animal diet, under the care of trained wardens. The rats were randomly divided into 2 groups as follows: HBO and control. The rats in the HBO group (n = 6) were placed into a 20-liter HBO chamber (2.5 atmospheres absolute [ATA], 25–26 °C with 100 % oxygen for 90 min per day. The rats received hyperbaric oxygen over a period of 7 days. They were not anesthetized and breathed spontaneously during HBO treatment. The rats in the control group (n = 6) were not given HBO, but were maintained in similar environment and food.

Tissue preparation for light microscopy
At the end of the study, animals were sacrificed by decapitation approximately 2 h after the end of 7 days of HBO exposure. The skin as well as all the soft tissues surrounding the nasal cavity were removed. Then, the bony-framework of the nasal cavity including the nasal septum was nibbled out with a bone-nibbler. The samples were fixed with neutral buffered formalin solution and decalcified with 5 % EDTA (Ethylenediaminetetraacetic acid). After preservation, nasal samples were directly dehydrated in a graded series of ethanol.
and embedded into paraffin wax. Five-micrometer sections were cut with a microtome (Rotary Microtome, Leica, RM 2265, Germany) and mounted on the coated slides. The sections were stained with Haematoxylin and Eosin (H-E), Periodic acid-Schiff (PAS) and Trichrome-Masson in order to be observed under a light microscope (Nikon Eclipse 80i). All morphological changes including inflammatory leukocyte infiltration and cellular hyperplasia, goblet cell hypertrophy and basal membrane thickness were noted. Semi-quantitative scaling of inflammatory leukocyte infiltration and cellular hyperplasia, goblet cell hypertrophy was performed. The intensity of these changes were graded from 0 to 3 (0: no infiltration or thickness, 1: faint, 2: moderate, 3: intense).

**Immunohistochemical staining**

Antigen retrieval process was performed in citrate buffer solution (pH 6.0) two times: first 7 min, and later 5 min boiled in a microwave oven at 700 W. The slides were allowed to cool to room temperature for 30 min and washed in distilled water for 5 min two times. Endogenous peroxidase activity was blocked in 0.1 % hydrogen peroxide for 15 min. Ultra V block (Cat. No: 85-9043, Invitrogen, Carlsbad, CA, USA) was applied for 10 min prior to the application of primary antibodies (E-cad antibody, mouse monoclonal, 1/200, Santa cruz) overnight. Secondary antibody (Cat. No: 85-9043, Invitrogen, Carlsbad, CA, USA) was applied for 20 min. Slides were then exposed to streptavidin-peroxidase for 20 min. Diaminobenzidine (DAB, Invitrogen, Carlsbad, CA, USA) was applied for 20 min. Slides were then exposed to streptavidin-peroxidase for 20 min. Diaminobenzidine (DAB, Invitrogen, Carlsbad, CA, USA) was used as a chromogen. Control slides were prepared as mentioned above but omitting the primary antibodies. After counterstaining with Hematoxylin, washing in tap water for 5 min and in distilled water twice for 5 min, the slides were mounted. The immunoreactivity of the pseudostratified epithelial cells of the nasal mucosa was assessed. Semi-quantitative scaling of immunoreactivity was carried out. The intensity of the staining was graded from 0 to 3 (0: no staining, 1: faint staining, 2: moderate staining, 3: intense staining).

**Statistical analysis**

Statistical analysis was performed with the Statistical Package for the Social Sciences for Windows (version 15.0, SPSS Inc.,Chicago, IL, USA). The Mann–Whitney U test was used for the statistics as indicated, and results were expressed as mean ± SD. P values below 0.05 were considered to indicate statistical significance.

**Results and Discussion**

The histopathological results from the present study showed significant differences in average histopathological scores between the group exposed and non-exposed to HBO (Table 1, Fig. 1 and Fig. 2). Exposure to HBO led to histopathological changes in the nasal mucosa of rats (Fig. 2). There were no histologic lesions observed in any of the control group animals. There were also no significant differences between the two groups for E-cadherin expression in immunohistochemical evaluation (Table 1, Fig. 3).

The treatment efficacy of HBO is based on two effects (10). The first is mechanical effect on bodily gases, the other is the incremental effect of blood partial oxygen pressure. Then, there are two major factors involved in HBO effect on the respiratory nasal mucosa: physical stimulation of the mucosa by increased atmospheric pressure and chemical stimulation of the tissues by 100 % O₂.

### Table 1

Comparison of HBO and control groups by means of E-cad (E-cadherin) expression and histopathological features

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>HBO</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>E-cad</td>
<td>1.66 ± 0.51</td>
<td>1.50 ± 0.54</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>0.16 ± 0.40</td>
<td>2.0 ± 0.63</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Goblet hypertrophy</td>
<td>0.0 ± 0.0</td>
<td>2.83 ± 0.40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leukocyte infiltration</td>
<td>0.33 ± 0.51</td>
<td>2.50 ± 0.54</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

![Fig. 1. Comparison of HBO and control groups by means of E-cad (E-cadherin) expression and histopathological features.](image)

![Fig. 2. Normal appearance of the epithelial layer and connective tissue areas in control group sections (a). Degenerative changes in epithelial cells were observed in certain cells. Goblet cells (g) showed expansion of the structure. In the connective tissue under the epithelium, some of the blood vessels were ruptured, and some of the erythrocytes (e) were observed in the free state (b). In the connective tissue located in the concha nasalis inferior, significant mononuclear lymphocyte infiltration (asterisks), dilation of blood vessels (d), and hemorrhage were observed. The irregularity and hyalinization in the Havers channels (Hc) were seen (c). In the area of the basal membrane, basal cell degeneration, and hyperplasia were seen. Connective tissue was infiltrated with mononuclear cells (asterisks), and erythrocytes were freely distributed (d). Significant expansion of goblet cells, among other hyperplastic (h) epithelial cells, was seen in the vacuolar structures (v) (e). Epithelial cells, hyperplasia (h), and thickening of the apical side of the cilia, shortening lengths of goblet cells also showed marked hypertrophy (hp). Under the epithelial cells, diffuse enlargement with infiltration of basal membrane was observed (f). Masson-Trichrome (a,d), H-E (b,c) and PAS (e,f).](image)
that samples of the turbinate mucosa of the hBo-treated chronic hBo therapy. In this study, animals were divided into three experimental groups submitted to a single (485-minute-long HBO session) or chronic HBO (30 daily sessions of 100-minute-long HBO), or used as controls. They did not observe any significant morphological changes after a single HBO treatment. However, they demonstrated that chronic HBO treatment causes minor changes in the rat nasal mucosa; they reflect a mild inflammatory response of the respiratory tract to the increase in pressure and in oxygen content induced by HBO. In our study, we used 7 days of HBO therapy. As there are no published reports with this therapy duration, our study provides additional knowledge by using this HBO therapy duration. However, we suggest that these changes are reversible histopathological interacting.

**Conclusions**

HBO treatment may produce some structural changes on the rat nasal mucosa. HBO treatment was found to cause mild inflammation in nasal mucosa and increasing thickness of the pseudostratified columnar epithelial layer of the nasal mucosa. However, further ultrastructural studies are required to confirm these effects.

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**REFERENCES**