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Investigating the Healing Effect of Hyperici Oleum and *Triticum vulgare* on Septal Perforation in Rats: A Preliminary Study

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ABSTRACT

Objective: The aim of this study was to investigate histopathologically the possible beneficial effects of hyperici oleum and *Triticum vulgare*, having anti-inflammatory, antioxidant, and antiproliferative properties, on the healing process of nasal septum perforation experimentally.

Methods: Circular perforations of 2 mm diameter were performed at the nasal septum of 32 rats. Intranasal administration of hyperici oleum (HO), *Triticum vulgare* (TV), and thiocilline (T) was carried out twice a day for 7 days. After the rats were sacrificed at the end of the seventh day, their nasal septum specimens were sent for histological examination. The groups were compared in terms of epithelial regeneration, presence of fibroblasts and inflammatory cells, and capillary density parameters.

Results: There was a statistically significant increase in the epithelialization mean scores in groups HO and TV compared to group P. When the groups were examined in terms of inflammation, a statistically significant increase was found in group HO. In terms of the fibroblast scores, a statistically significant increase was seen between group P and groups HO and T. The vascularization mean scores both in group HO and group TV were statistically significant when they were compared with groups C and P.

Conclusion: The present study provides clues for preferring the use of TV and HO as a healer for the perforation area in the treatment of septum perforation. Based on the findings, widening the experiment and including additional parameters for understanding the healing mechanism will contribute to the clarification of the healing mechanism and thus to the development of the treatment options.

Keywords: Hyperici oleum, *Triticum vulgare*, nasal septal perforation, nasal septum, wound healing

Introduction

Nasal septum perforation (NSP) is defined as anatomical disorder that develops in the cartilage and/or bone structure after necrosis occurring in mucoperichondrium and mucoperiosteum for any reason in the nasal septum, that is formed of the mucosa, cartilage, and the bone, and causes the formation of a bond between both nasal cavities.[1,2] Even though NSP can be seen in the anterior, posterior, and superior regions of the septum, the most frequent localization is the anterior (92%) section of the septum since it is more exposed to trauma.[2]

The etiology of NSP may include trauma (septal fracture, septal hematoma, nasal foreign bodies and nose piercing, continuous nose picking, etc.), procedures causing iatrogenic trauma (submucosal resection (SMR) septoplasty, functional endoscopic sinus surgery, septal cauterization, nasotracheal intubation etc.), long-term intranasal drug use, cocaine use, some systemic drugs, and besides occupational exposure, inflammatory (vasculitis, collagen vascular diseases, sarcoidosis, wegener granulomatosis), infectious (tuberculosis, syphilis, lepromatous leprosy, mucormycoses, diphtheria, acquired immunodeficiency syndrome, etc.), and malignant diseases.[2,3] Systemic diseases often play a role in the etiology of NSPs that are localized in the posterior and superior regions and they are clinically asymptomatic. In the etiology of NSP in the anterior region, the most common cause is trauma and the symptoms observed in its clinics can be specified as persistent or recurrent sinonasal symptoms, nasal congestion, epistaxis, crusting, pain in the facial area, and whistling sound.[4]

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Hypericum perforatum L. (Hypericaceae), known as its popular name St. John's wort, has a rich historical background and is one of the oldest and most studied herbs. For centuries, *H. perforatum* L. has been used to treat various diseases in phytotherapy medicine such as depression, aging memory disorder, quitting smoking, rheumatism, irritable bowel syndrome, bacterial infection, wound healing, and skin lesions.[5] Numerous bioactivities and applications of *H. perforatum* L., including antibacterial, antiviral, anti-inflammatory, antidepressant, anxiolytic, wound healing, and analgesic effects, are included in the literature[6,7] Hypericum oleum (HO), that is, a fat macro of *H. perforatum* flowers, is widely used as a traditional drug for skin ulcerations and burns in Bosnia and Herzegovina, Turkey, and Serbia.[8] The studies that have started to take place in the literature in recent years show that the antibacterial effect of HO reduces the size of external wounds and its healing time.[9]

Triticum vulgare (TV) is widely used in traditional medicine due to its properties accelerating tissue regeneration. *T. vulgare* extract is present in some pharmaceutical formulations that are used in the treatment of decubitus ulcers, skin lesions, and burns. Also, various studies have revealed that the TV extract may have potential anti-inflammatory properties. *Triticum vulgare* significantly accelerates the tissue healing process and shapes the connective tissue by stimulating the chemotaxis of the cells and fibroblast activation.[10-14]

Local inflammatory reaction starts when NSP develops for any reason. The cells taking part in inflammation secondary to this inflammation are infiltrated in submucosal area and lead to losses in mucosal and submucosal layers and decreases in vascularization. These histopathological changes cause ischemic changes and necrosis in the cartilage. If an irregular regeneration or repair occurs in the mucous layer located at the margins of perforation, this area is covered with the atrophic epithelial layer and NSP develops. Therefore, wound healing plays an important role both in the formation and treatment of NSP.[15] Although NSP generally progresses asymptomatically, it causes significant morbidity in symptomatic cases. It may cause a decrease in the quality of life of the person especially in symptomatic cases. In such cases, conservative medical treatments such as nasal irrigation and moisturizing oil drops or surgical treatments according to the severity of the manifestation should be applied. A wide range of methods are described in surgical treatment. However, successful repair of these perforations via surgical methods involves difficulties even for experienced surgeons.[16] This situation has caused to emphasize on alternative treatment approaches in the treatment of NSP in recent years.

Main Points

- Wound healing plays an important role both in the formation and treatment of nasal septum perforation.
- The antibacterial effect of hyperici oleum reduces the size of external wounds and its healing time.
- *Triticum vulgare* (TV) is widely used in traditional medicine due to its properties accelerating tissue regeneration.
- Wound healing plays an important role both in the formation and treatment of nasal septum perforation.
- Despite the limitations, we were able to reveal that HO and TV administered histopathologically after perforation accelerated wound healing, activated epithelialization, stimulated the connective tissue, had an anti-inflammatory effect, increased collagen fibers, and stimulated the formation of new blood vessels.
- Consequently, the present study provides clues for preferring the use of TV and HO as a healer for the perforation area in the treatment of septum perforation.

We consider that HO (St. John's wort) and TV (wheat germ oil) can be evaluated as a cheap option in the treatment of NSP. Therefore, the aim of the present study was to investigate histopathologically the possible beneficial effects of HO (St. John's wort) and TV (wheat germ oil), having anti-inflammatory and antioxidant properties, on the healing process of nasal septum perforation experimentally induced in rats.

Methods

Experimental Animals

The trials were conducted based on the National Institute of Health (NIH) Guide for the care and use of Laboratory Animals (NIH Publications No. 80-23 Revised 1996). For the protocol of the study, approval of the Institutional Review and Animal Ethics Use Committee of Sivas Cumhuriyet University School of Medicine was obtained. The study was conducted in accordance with the accepted guidelines on the care and use of laboratory animals (Date: September 3, 2019; Decision number: 65202830-050.04.04-30421.03.2019).

This randomized experimental trial was carried out based on the aforementioned protocol. Female, 16-18-week-old Wistar albino rats having an average body weight of 230 ± 10 g were used in this study. The animals were kept under standard laboratory conditions (12 h light:12 h darkness cycles, 24 ± 2°C, 35%-60% humidity).

Fifty animals were anesthetized with 3 mg/kg xylazine subcutaneous (SC) and 90 mg/kg ketamine HCL SC and then their nasal cavity and septum were selected after they were examined using a surgical microscope (OPMİ Vario; Zeiss, Jena, Germany). The animals with nasal pathologies (for example, infection in the nasal cavity, purulent discharge and pathology of the nasal septum mucosa) were excluded from the study. Forty rats having completely healthy nasal mucosa were included in the study.

The rats (n = 40) were assigned to four groups through randomization [to the researchers decided to include 8 rats into each group considering $\alpha = 0.05$, $\beta = 0.10$, $(1 - \beta) = 0.90$; the power of the test was found to be $P = .9039$] (Table 1).

It was planned to exclude rats dying during the study due to any reason in experimental protocol, but no rat died.

Drug and Chemicals

Thiocilline (bacitracin + neomycin sulfate, Abdi İbrahim İlaç, Turkey), TV (Rosece Wheat germ oil, Orgamyra Trade. LTD. Sti.,Ankara, Turkey, Rosece, no: 117, 30 mL), and HO (Zade Vital, Zade Global Inc. Konya, Turkey 100 mL) were obtained.

Table 1. The Study Groups

Group Name	Explanation of Study Groups (n)
Group C	Control group with intact mucosa without perforation (n=8)
Group P	Perforation left to spontaneous healing and no medication was applied (n=8) (negative control group)
Group T	Intranasal thiocilline was administered twice a day (morning–evening)/7 days, (n=8) (positive control group)
Group HO	Intranasal HO was administered twice a day (morning–evening)/7 days, (n=8)
Group TV	Intranasal *Triticum vulgare* was administered twice a day (morning–evening)/7 days, (n=8)

C, control; HO, hyperici oleum; P, negative control; T, thiocilline; TV, *Triticum vulgare*.

Operation Procedure

The rats (n=32) were anesthetized with subcutaneous injections of 90 mg/kg ketamine hydrochloride (Ketalar®, Pfizer Turkey, Istanbul, Turkey) and 3 mg/kg xylazine hydrochloride (Rompun®, Bayer, Istanbul, Turkey). Approximately 10 minutes after administration of the anesthetic agent, a 2 mm posterior and 2 mm diameter circular perforation behind the columella was performed using a 2 mm width standard circular perforator on the nasal septum mucosa in each experiment. Metamizole 20 mg/kg intramuscular (Novalgin, 2 mL; Sanofi, Paris, France) was used as a pain killer. The same surgeon (A.B.) followed the necessary sterilization conditions while performing all surgical procedures.

Study Protocol

- Group C (control group, n=8): No surgical procedure on the nasal septum mucosa of the rats and no medication was applied.
- Group P (negative control group, n=8): The nasal septum mucosa of the rats was perforated and left to spontaneous healing and no medication was applied.
- Group HO (n=8): The nasal septum mucosa of the rats was perforated and HO (St. John's wort) was applied intranasally to the nasal cavity twice a day.
- Group T (n=8): The nasal septum mucosa of the rats was perforated and applied to thiocilline intranasally in the nasal cavity twice a day.
- Group TV (n=8): The nasal septum mucosa of the rats were perforated and TV was applied intranasally to the nasal cavity twice a day.

All the drug administrations were performed by the same person in the perforation in the left nasal cavity twice a day for 7 days in the same time period.

At the end of 7 days, all rats were sacrificed with intraperitoneal injections of pentothal sodium (200 mg/kg). From the nasal septum, both septal mucosa and cartilage excision were performed through a 5 × 5 mm through-cut excision covering the experimental perforation border. Tissue samples of the groups were put in containers without group names. After each of the biopsy materials was received, it was subjected to histopathological examination.

Histopathological Examinations

One pathologist (Z.D.S.I.) who was blind to the samples performed the histological analyses. Tissue samples taken were fixed for 24-48 hours by coding in 10% buffered neutral formalin in the containers without the group names. Then, the tissue was softened by keeping it in a decalcification solution for 30 days in a controlled manner. Softened tissues were passed through a tissue follow-up and paraffin was blocked. From the paraffin blocks, 3-5 µm sections were taken and evaluated by using a light microscope (Olympus BX51, Tokyo, Japan) after staining with hematoxylin–eosin (H&E). A digital camera and auxiliary equipment (Nikon USB (H) EXT 1/0, Nikon Instruments Inc., Tokyo, Japan) were used with the microscope to obtain digital images of the sections.

According to the histopathology of normal septum, histopathological parameters were evaluated as follows: Regeneration and degeneration of the perforation epithelium, the presence of fibroblast and collagen, density of the capillary vessels, the presence of acute and chronic inflammatory cells, and amount of eosinophil. This evaluation was scored and evaluated statistically (Table 2).[15]

Statistical Analysis

The data were analyzed by using the Statistical Package for the Social Sciences Statistics (IBM SPSS Corp.; Armonk, NY, USA)

Table 2. Histopathological Grading and Scoring of Septal Perforation Healing[15]

Histopathological Evaluation of Septal Perforation Healing				
Grading and Scoring	Epithelialization	Inflammatory Cell Density (InfCD)	Fibroblast Cell Density	Capillary Density
0	No epithelialization, fibrin coverage in the wound area	No inflammatory cells	No fibroblast cell	Very few new vessels
1	Initiation of epithelialization, ~30%	Focal and few cells	Few fibroblast cells	Few new vessels
2	Epithelial covering, 30%-60%	Medium number of cell densities	Medium number of fibroblast cell density	Moderate new vessels
3	Epithelial covering, 50%-85%	Spreading and many cell density	Many fibroblast cell density	Lots of new blood vessels
4	Epithelial covering, 85%-100%	Prevalent cell density	Frequent cell density	Many new blood vessels

Table 3. Comparisons of Histopathological Findings Among the Groups

Parameter	Groups	Mean ± SD (Min-Max)	Median ± IQR (Q1-Q3)	*P*
Epithelialization	Group C	3.75 ± 0.46 (3-4)	4 ± 0.5 (3.5-4)	<.001
	Group P	1.375 ± 0.52 (1-2)	1 ± 1 (1-2)	
	Group TV	2 ± 0.53 (1-3)	2 ± 0 (2-2)	
	Group HO	2.875 ± 0.83 (2-4)	3 ± 1.5 (2-3.5)	
	Group T	3.5 ± 0.76 (2-4)	4 ± 1 (3-4)	
Inflammation	Group C	2.375 ± 0.52 (2-3)	2 ± 1 (2-3)	.023
	Group P	3.5 ± 0.76 (2-4)	4 ± 1 (3-4)	
	Group TV	2.75 ± 0.89 (2-4)	2.5 ± 1.5 (2-3.5)	
	Group HO	3.375 ± 0.74 (2-4)	3.5 ± 1 (3-4)	
	Group T	2.25 ± 1.04 (1-4)	2 ± 1.5 (1.5-3)	
Fibroblast	Group C	2 ± 0.53 (1-3)	2 ± 0 (2-2)	.017
	Group P	1.5 ± 0.53 (1-2)	1.5 ± 1 (1-2)	
	Group TV	1.875 ± 0.64 (1-3)	2 ± 0.5 (1.5-2)	
	Group HO	2.75 ± 0.89 (2-4)	2.5 ± 1.5 (2-3.5)	
	Group T	3 ± 0.76 (2-4)	3 ± 1 (2.5-3.5)	
Vascularization	Group C	1.875 ± 0.64 (1-3)	2 ± 0.5 (1.5-2)	<.001
	Group P	1.875 ± 0.64 (1-3)	2 ± 0.5 (1.5-2)	
	Group TV	3 ± 0.53 (2-4)	3 ± 0 (3-3)	
	Group HO	3.625 ± 0.52 (3-4)	4 ± 1 (3-4)	
	Group T	3.75 ± 0.46 (3-4)	4 ± 0.5 (3.5-4)	

P values show the results of the Mann–Whitney *U*-test.
Results were evaluated at a significance level of $P < .05$.
C, control; HO, hyperici oleum; P, negative control; T, thiocilline; TV, *Triticum vulgare*.

for Windows, version 22.0. Since the parametric test assumptions could not be performed in the evaluation of the data (n < 30), Kruskal–Wallis test was used for the comparison of data obtained from more than two independent groups, and Mann–Whitney *U*-test was used to find the groups making difference when a significance decision was given in the analysis result. Level of significance was set at the value of 0.05.

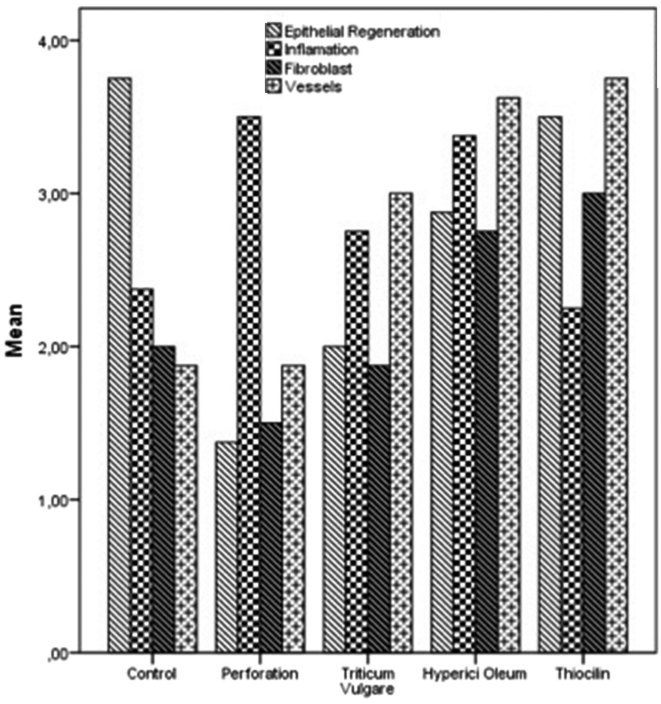
Results

When the groups were evaluated in terms of epithelialization in the tissue samples obtained in the study, no statistically significant difference was found between groups C and T ($P > .05$). In terms of the epithelialization mean scores, the difference between groups TV and HO and group C was statistically significant and it was lower in groups TV and HO ($P < .05$). However, when the epithelialization mean scores in group P were compared, a statistically significant increase was seen in both groups ($P < .05$). When the results obtained were evaluated statistically, if an application other than thiocilline would be preferred for the primary purpose of epithelialization increase, the first option should be HO ($P_t = .129$, $P_c = .030$) and the second should be TV application ($P_t = .003$, $P_c = .001$).

When the groups were examined in terms of inflammation, there was no statistically significant difference between groups C, T, and TV ($P < .05$). A statistically significant increase was found in group HO ($P < .05$). It was observed that thiocilline and TV provided nearly total recovery. A statistically significant difference was not determined between groups P and HO ($P < .05$).

When the groups were compared in terms of the fibroblast mean scores histopathologically detected in the perforation area, a statistically significant difference was found in group T compared to group C ($P < .05$). No statistically significant difference was found between groups TV and C and group HO ($P > .05$). However, in terms of the fibroblast scores, a statistically significant increase was seen between group P and groups HO and T ($P < .05$). When these results obtained were evaluated statistically, if an application would be selected other than thiocilline in terms of fibroblast, this should be the HO application ($P_t = .502$, $P_c = .069$).

No significant difference was found between groups C and P when a statistical evaluation in terms of the vascularization mean scores was conducted ($P > .05$). The highest vascularization mean score was seen in group T [3.75 ± 0.46 (minimum–maximum: 3-4)] and this difference was statistically significant when groups C and P were compared ($P < .05$). In Group HO [3.625 ± 0.52 (min-max: 3-4)], vascularization mean score was similar to group T ($P > .05$) and this difference was statistically significant when compared with groups C and P ($P < .05$). When the vascularization mean scores of group TV were



Graphic 1. Comparisons of histopathological findings among the groups.

compared with mean scores of groups C and P, the observed difference was statistically significant ($P < .05$). However, it was lower than the mean scores determined in groups T and HO. Table 3, Graphic 1, and Figure 1A-E shows the histopathological findings of each group.

Discussion

Nasal septum perforation develops as a result of the necrosis occurring in the cartilage and/or bone tissue following the damage caused by any reason both in mucopericondrium and mucoperiostium.[15] The main cause of the NSP is iatrogenic ones like nasal surgery and self-inflicted trauma. Many different surgical techniques are applied in NSP repair in which grafts and local flaps are used; however, it should not be ignored that there are difficulties in each of these techniques. The skills of the surgeon are at the top of these difficulties; the wound healing process and the degree of deterioration in nasal physiology also have an important role in the treatment of NSP.[17]

Wound healing observed in tissue defects occurring after the surgery or trauma is a well-organized process. At first, inflammation begins in the wound area and this is followed by the proliferation phase. Maturation (remodeling) occurs in the final phase. It is known that increased oxidative stress, impaired tissue feed, and increased tissue destruction secondary to the

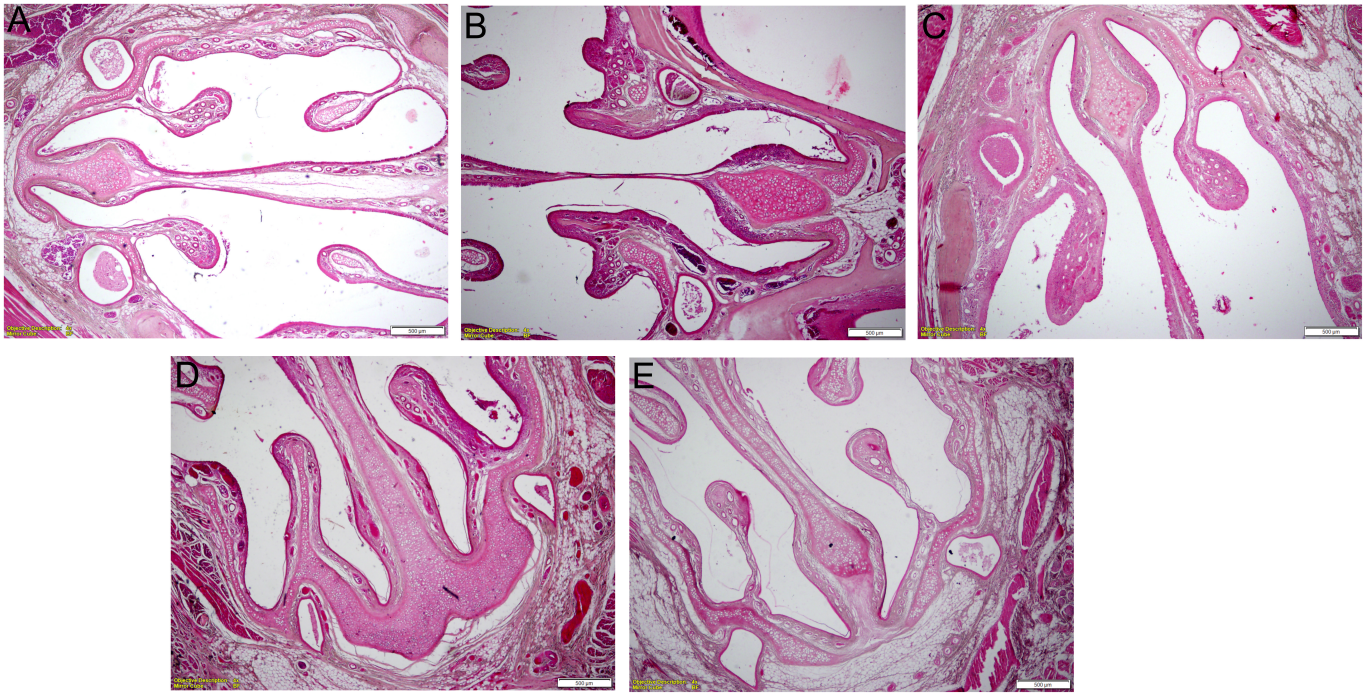


Figure 1. A-E. (A) Group C: False multilayer goblet epithelium lining the nasal cavity, glands in the subepithelial connective tissue, blood vessels, and nerve fibers show regular morphology. (B) Group P: Perforation areas in the nasal septum epithelium are clearly observed in and under the epithelium. Especially, the loss of serous glands in the subepithelial connective tissue, increase in the inflammatory cell, and lack of vascular structures are strikingly observed. (C) Group T: Epithelialization in the perforation areas in nasal septum occurred similarly as in group C. There were inflammatory cells under the epithelium, and the gland structures were partially shaped in the subepithelial connective tissue, a few new blood vessel areas were identified. (D) Group HO: It can be seen that epithelialization was mostly completed in the perforation areas around the nasal septum. The number of the inflammatory cells in subepithelial lamina propria decreased and the number of the blood vessels increased. (E) Group TV: The epithelium in the perforation areas in the epithelium surrounding the nasal septum was irregular and cell increase and epithelialization were observed in the perforated epithelium. Inflammatory cells in the subepithelial connective tissue reduced compared to group P but they were still present compared to group C. Blood vessels inside the connective tissue were observed.

increase in inflammation have a negative effect on the wound healing process. Also, reduction of inflammation, protection of the tissues from infection, induction of cell proliferation for reconstruction, and reduction of free oxygen radicals also contribute positively to provide optimum wound healing.[15]

The factors affecting the healing process and regeneration of septal mucosal defects occurring due to various reasons have not been still revealed exactly. This directs the scientists to investigate the effects of different substances with known anti-inflammatory and antioxidant properties that accelerate wound healing as an alternative and/or a combination for the surgical treatments in the treatment of patients with NSP.

Çakan et al[15] examined the potential beneficial effects of curcumin, with known anti-inflammatory, antioxidant, antiproliferative, and positive effects on wound healing that is derived from the roots of turmeric, on intranasal topical application and wound healing of nasal septum mucosa. Their results revealed that topical curcumin administration has a positive effect on wound healing in animal model of NSP and can be used as a safe and effective medical agent to prevent the development of NSP. Aksoy et al[18] evaluated the effects of N-butyl cyanoacrylate, which is a tissue adhesive, on the nasal septal tissues in experimental rats in terms of bleeding and hematoma formation in the first 24 hours and histopathological changes in 4th and 12th postoperative weeks. At the end of the study, it was revealed that the application of N-butyl cyanoacrylate had a positive contribution on inflammation, bone and cartilage formation, and wound healing besides reducing the hematoma formation. Khalmuratova et al[19] evaluated the postoperative systemic dexamethasone application on nasal wound healing histopathologically on different days after the trauma. In steroid administration in rats, less subepithelial edema formation and epithelial irregularities were observed in the early phase of wound healing, while ciliary and goblet cell indices were lower. Thus, they highlighted that dexamethasone application may delay the regeneration of ciliary and goblet cells. It was also found that dexamethasone application did not contribute to the subepithelial tissue thickness and epithelial thickness. Choi et al[20] investigated the effects of the application of silastic sheet, hyaluronic acid, and both on the regeneration process of mucosal defect that surgically formed in the nasal septum of rabbits. As a result of the histopathological evaluation performed in the fifth week of the present study, no significant difference was found in terms of the number of ciliary cells, mucous and epithelial thickness at the wound site. However, it was determined that although they were not statistically significant, they did not increase mucosal regeneration when hyaluronic acid was applied alone, but the healing of the mucosa was faster when applied in combination with the silastic sheet. Manciula et al[21] investigated the effect of astaxanthin, which is a strong antioxidant, on the healing process of the nasal mucosa in the postoperative period. They showed that astaxanthin had a positive effect on the mucosal tissue as well as reducing fibrosis and synechia formation in the wound area. Apart from these studies, there are studies investigating the effects of topical steroids, carboxymethylcellulose, and chitosan on the healing processes of the nasal mucosa.[22-23] However, no exact and reliable results could be obtained in any of them.[24]

It is known that TV and HO have positive effects on the healing of scar tissue in different regions. TV accelerates the wound healing process by reducing the expression of the inflammation mediators such as interleukin 6, tumor necrosis factor α, prostaglandin E2, and nitric oxide.[25] *Hypericum perforatum* L. and its components have an anti-inflammatory effect by inhibiting the protein kinase C activity, arachidonic acid, and leukotriene B4 release and inhibiting effect on the lymphocytic reaction in the epidermis and the proliferation of the local T cells. Due to these properties, it contributes positively to the wound healing processes.[26] However, no study investigating the effects of TV and HO topical applications on the nasal mucosa could be found in the literature. Thus, in the present study, the potential useful effect of HO and TV having anti-inflammatory and antioxidant properties on the healing of NSP was investigated.

As known, wound healing is a dynamic process and the migration of different cells such as fibroblasts, to the damaged area is an important stage of this process.[27] Fibroblasts play an important role in wound healing with the growth factor, cytokines, collagen, and other extracellular matrix components they release. Additionally, fibroblast migration and proliferation have important roles in the healing process by initiating the proliferative phase.[28] When the results obtained were evaluated, the epithelialization and fibroblast mean scores showed that they were lower in TV and HO groups compared to the experimental rats in the control groups but higher than group P.

The inflammatory response after the tissue damage has important roles in both normal and pathological healing.[29] Pathological healing refers to the observation of a greater number of inflammatory cells than in the normal healing phase during microscopic examination**.** When the results obtained were evaluated, it was seen that the inflammation mean scores detected in the TV application were similar to the mean scores of the control groups, but the HO application was similar to the mean scores of group P. The fact that TV application caused less inflammation than HO application may suggest that TV should be preferred more in NSP treatment. However, we consider that stating an absolute judgment on this issue would not be appropriate since cells and/or inflammation markers having a role in wound healing were not assessed in the present study.

Neovascularization has a basic effect from onset of skin injury until the completion of the wound remodeling.[30] The evaluation made in terms of vascularization mean scores has revealed that TV and HO applications caused an increase in vascularization and this increase was higher in HO application.

The direct effect of HO on NSP was not investigated. However, in a study investigating the potential effect of *H. perforatum* on wound healing, it was emphasized that fibroblasts, collagen fibers, and blood vessels showed a significant increase during the process of wound healing, and accelerated healing.[9] Similarly, in another study, the effect of HO extract on skin wounds was investigated and it was expressed that it activated the fibroblast cells in the HO-treated groups and the density of the collagen fibers was significantly higher than other groups.[31] In the study conducted by Gunpinar et al[32] in 2020 to examine excisional palatal wound healing in rabbits, they expressed that HO accelerated the factors regulating the new vessel formation and increased the wound healing. Our study also exhibits

similarities with the histological characteristics of the wound healing model used in the study conducted by Gunpinar et al.

In an in vitro study conducted with TV extract, it was emphasized that it biologically activated human endothelial cells.[33] In another study, it was reported that TV accelerated the growth of fibroblast cells in an in vitro environment and accelerated the wound healing if it was applied to in vivo wound areas.[34] Through different clinical studies, it is also known that TV extracts are effective on the fibroblasts activating and supporting the tissue regeneration and healing process.[11-13] It has been documented that there is an anti-inflammatory effect that facilitates the transition from the inflammatory phase to the regenerative phase during the healing process. Additionally, there are studies indicating that one significant factor in this process is the stimulation of matrix metalloproteinases, which promote the development of connective tissue, consequently expediting wound healing and enhancing the healing process by increasing the production of anti-inflammatory cytokines[35] However, there is no study evaluating its effect on the perforated areas in septal perforations.

According to these results, we estimated that the present study has contributed to the literature because it is the first study that histopathologically investigates the possible useful effects of TV and HO, known to have positive effects on wound healing at different regions, regarding the healing process of NSP in the databases that can be accessed. However, it cannot be neglected that the study has some limitations. These limitations can be listed as follows: low number of rats, execution of the histopathological evaluation after a short-term application and the failure to evaluate the histopathological changes that may be caused by shorter or longer applications, and the failure to assess the parameters playing a role in wound healing such as growth factors, inflammatory cytokines, and free oxygen radicals. Additionally, another limitation of the present study is that only the regeneration of the mucosa was histopathologically evaluated and functional healing could not be assessed. When all these limitations were considered, we believe that it would be appropriate to accept the present study as an initial study on this matter. This is because the results of the present study suggesting that HO and TV treatments may have positive effects on the healing process of NSP are remarkable.

Despite the limitations, we were able to reveal that HO and TV administered histopathologically after perforation accelerated wound healing, activated epithelialization, stimulated the connective tissue, had an anti-inflammatory effect, increased collagen fibers, and stimulated the formation of new blood vessels. Consequently, the present study provides clues for preferring the use of TV and HO as a healer for the perforation area in the treatment of septum perforation. Based on the results of the present study, widening the experiment and adding additional parameters for understanding the healing mechanism will contribute to the clarification of the healing mechanism and thus the development of treatment options.

Ethics Committee Approval: This study was approved by Ethics Committee of Sivas Cumhuriyet University, (Approval No: 65202 830-050.04.04-30421.03.2019, Date: September 3 2019).

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Peer-review: Externally peer-reviewed.

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