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A Novel Effect of *Allium tuncelianum* Extract: Topical Application Improves Wound Healing in a Nasal Septal Perforation Rat Model

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ABSTRACT

Objective: The objective of the current study was to investigate the influence of *Allium tuncelianum* (AT) extract on wound healing in a nasal septum perforation (NSP) model.

Methods: Twenty-two healthy male Sprague–Dawley rats were included in this study and separated into two groups. A standardized NSP was created in each subject. A 0.09% saline (control group) and the 25% AT solution (study group) were delivered intranasally daily for 14 days. At the end of the experiment, the subjects were sacrificed and the septa were excised for histopathological investigation. The macroscopic closure rate of NSP, intranasal pH, counts of acute inflammatory cells, eosinophil, fibroblast, and giant cell, epithelial regeneration and degeneration, vascularization, granulation formation, collagen density, cartilage regeneration, and degeneration were examined. The obtained data were analyzed statistically.

Results: The macroscopic closure rate ($p = 0.006$), the fibroblast number ($p = 0.003$), vascularization ($p = 0.003$), collagen density ($p = 0.044$), and granulation tissue amount ($p = 0.022$) were found to be significantly higher in AT group. However, the acute inflammatory cells count was significantly lower in AT group ($p = 0.031$) ($p < 0.05$).

Conclusion: The topically delivered AT extract may improve wound healing in an experimental NSP model. Consequently, the local application of AT might be promising to prevent the formation of NSP.

1 | Introduction

The term "wound" is described as the corruption of the normal anatomy and function of tissue due to trauma [1]. Wound healing refers to the dynamic, sequential coordinated, and complex physiological reaction that arises to renovate the functional anatomy disrupted by trauma [1, 2]. The process comprises four consecutive phases: hemostasis, initiated instantly post-trauma; inflammation, commencing shortly thereafter; proliferation, beginning in the subsequent days; and remodeling, which can extend over several years [1, 2].

Nasal septum perforation (NSP) is defined as a full-thickness loss of the septum, which is composed of bone, cartilage, and connective tissue, and covered by mucosa [3]. The incidence of NSP is higher in regions with prevalent intranasal drug use; however, large-scale population studies have reported an incidence of 1% or lower [3–5]. The most prevalent cause is iatrogenic, typically

arising during or after surgical procedures when tears occur in the mucosa that covers the region of excised cartilaginous or bony structures [3–5]. When damaged mucoperichondrial flaps of the nasal septum fail to self-heal and close the defect, the surrounding area may be covered with atrophic mucosa, resulting in permanent perforation [6]. Hence, the wound healing process significantly influences the formation of permanent perforation, optimal wound healing is necessary to prevent its occurrence [5, 6].

Substances that reduce oxidative stress, suppress inflammation, protect against harmful microorganisms, reduce infection risk, increase blood flow, and nutritional support, and possess antioxidant, anti-inflammatory, antimicrobial, angiogenetic, and proliferative properties expedite the stages of wound healing and improve this process [6, 7].

Allium tuncelianum (AT) (Kollman) Özhatay, Matthew and Şiraneci is an endemic *Allium* species found in the central-eastern regions of Türkiye. It bears resemblance to garlic (*Allium sativum* L.) in plant architecture, odor, and taste, and is considered to be the wild ancestor of garlic [8–10]. Previous studies have demonstrated that phytochemical molecules found in its composition possess antioxidant, antibacterial, antidiabetic, and anticarcinogenic properties [9–11]. However, there are no studies in the literature examining its effects on wound healing.

The objective of the current study was to investigate the influence of topical application of AT on wound healing in a rat model of NSP, employing macroscopic and histopathological analyses.

2 | Materials and Methods

The current research was conducted at the Bezmi Alem Vakıf University (BAVU) Experimental Animal Laboratory following approval from the BAVU Animal Experiments Local Ethics Committee (approval no: E7728, date: September 14, 2022). All procedures were performed by the ARRIVE guidelines 2.0.

2.1 | Subjects and Study Groups

Healthy male Sprague–Dawley rats, each weight 200–300 g and aged 9–10 weeks, were included in the current study. The sample size was determined as 22, based on the study by Ceylan et al. [12] utilizing a 95% confidence interval and a 5% margin of error. The subjects were maintained under standardized laboratory circumstances with a temperature of 22°C ± 2°C and a 12:12-h dark–light cycle. They were sheltered at four per cage and fed with standard pellet bait and tap water *ad libitum*.

2.2 | Drugs

In this study, the species identification of AT samples purchased from local vendors in Tunceli was performed by two experienced botanists. The underground parts of the plant were cut into small pieces, weighed, and finely powdered. These were then subjected to maceration in water at room temperature for 8 h daily over the course of 3 days. The resulting total extract was filtered and lyophilized until completely dry. The yield was determined to be 30%. The resulting dried plant extract was preserved in a fridge at 4°C. A 25% solution of AT extract was prepared by dissolving the extract in sterile distilled water (Polifarma, Türkiye). Saline (0.9%) (Turkfleks, Türk İlaç, Türkiye) was utilized in the control group. Saline and AT solutions were preserved in a fridge at 4°C during the study.

2.3 | Experimental Design

Following anterior rhinoscopic examinations the rats ($n = 22$) were divided into two at random: the AT (study) and saline (control) groups.

2.3.1 | A Standardized Nasal Septum Perforation Model

All subjects were anesthetized with ketamine hydrochloride at 45 mg/kg (Ketalar, Pfizer, USA) and xylazine hydrochloride at 5 mg/kg (Basilazin 25 mL 2% vial, Bavet) intramuscular injections. Following the procedure, the subjects were allowed to breathe spontaneously. A waiting period of 10 min was observed following the induction of anesthesia to ensure adequate depth. The depth of anesthesia was assessed by evaluating the finger pinch response and limb withdrawal reflex. The rats were positioned appropriately. A NSP, approximately 2 mm in diameter and 3 mm posterior to the columella, was established through the right nostril utilizing a cannula (B-CAT 2 IV Cannula 14G; 2.2 × 45 mm, Bıçakçılar, Türkiye) (Figure 1).

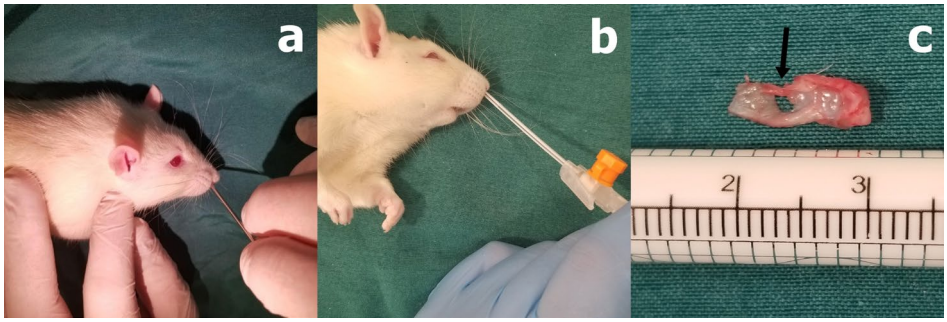

FIGURE 1 | (a) The creation of nasal septum perforation utilizing a cannula. (b) The intranasal application of *Allium tuncelianum* solution. (c) The macroscopic evaluation of the perforation.

2.3.2 | Drug Administration

After the perforation procedure, 2mL of solution was administered to each subject, divided equally between the nostrils (Figure 1). The solutions were applied once daily by the same person at the same time for 14 consecutive days. At the conclusion of the study, 14th day after surgery, subject euthanasia was implemented with an intraperitoneal injection of 100 mg/kg pentobarbital (Penbital, Bioveta, Czechia).

2.4 | Macroscopic and Microscopic Examinations

Subject weights (SW) and intranasal potential of hydrogen (pH) values were determined at the beginning and end of the experiment. A litmus pH test strip was placed on the nasal septum and waited for 10 s. The resulting color change was evaluated with the color scale. At the study termination, the nasal septum of each subject was surgically excised. The sizes of the perforations on the nasal septum were determined blindly utilizing a surgical ruler. The maximum diameter of the perforation was measured, and the initial perforation diameter was assumed to be 2.2 mm for evaluation (Figure 1). The macroscopic evaluations were performed blindly by the same person. The closure of the perforation was macroscopically scored into four categories: 0: enlarged (> 2.2 mm), 1: unchanged, 2: reduced (< 2.2 mm; > 0), and 3: closed. After macroscopic examination, each dissected specimen was individually placed into separate containers without assigning group names and fixed in a 10% standard formaldehyde solution for subsequent histopathological analysis.

Paraffin-embedded blocks were prepared in the pathology department. Sections of 3 μm thickness were cut from these blocks. Masson trichrome and Hematoxylin–eosin (H&E)-stained sections were evaluated under brightfield microscopy at high magnification (Nicon, Eclips E 600, Tokyo, Japan). Histopathological evaluations were conducted blindly by an expert pathologist with decade of experience. Histopathologic ally, acute inflammatory cells count, epithelial regeneration, epithelial degeneration, fibroblast count, vascular density, granulation tissue density, cartilage degeneration, cartilage regeneration, collagen density, eosinophil count, and giant cell presence were evaluated (Figure 2). This assessment was subjectively scored as follows: 0 (none), 1 (mild), 2 (moderate), and 3 (intense) [5, 6].

2.5 | Statistical Analysis

The minimum sample size was determined using the G*Power software. Statistical analyses were carried out using the Statistical Package for the Social Sciences (SPSS) 23 (IBM Corp, USA). Mean (mean), standard deviation (SD), and median values

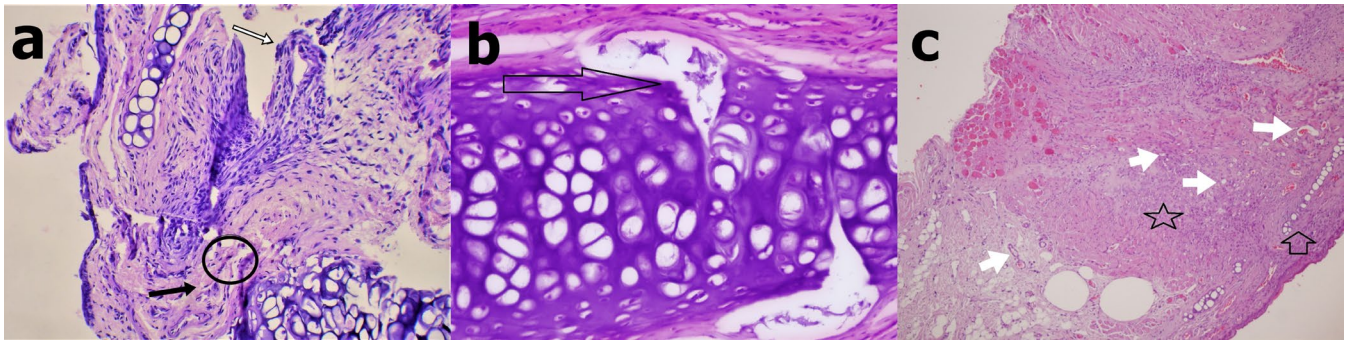

FIGURE 2 | (a) The increased inflammation (black arrow), cartilage (circle), and epithelial degeneration (white arrow) in the control group (The circle indicates the perforated nasal septum, H&E, ×100). (b) Cartilage degeneration in the control group (arrow) (H&E, ×400). (c) The granulation tissue formation (asterisk), neovascularization (white arrow), and cartilage regeneration (black arrow) in the study group (H&E, ×200).

TABLE 1 | The evaluation of subject weight and intranasal potential of hydrogen parameters.

Parameter		Control group	Study group	*p**
		Mean ± SD (median)		
Subject weight (g)	Initial	276.181 ± 17.899 (275)	276.363 ± 19.377 (275)	0.974
	Final	197.909 ± 15.751 (300)	296.818 ± 20.405 (300)	0.692
	*p***	0.003[a]	0.003[a]	
Potential of hydrogen (pH)	Initial	7.136 ± 0.233 (7)	7.272 ± 0.261 (7.5)	0.204
	Final	7.227 ± 0.261 (7)	7.318 ± 0.252 (7.5)	0.403
	*p***	0.317[b]	0.564[b]	

[a]$p < 0.05$.
[b]$p > 0.05$.
*Mann Whitney U test, $p > 0.05$.
**Wilcoxon Signed Ranks test.

were calculated. The comparisons were performed utilizing the Mann–Whitney U and Wilcoxon Signed Rank tests. A significance level of $p < 0.05$ was considered statistically significant.

3 | Results

The weights and intranasal pH values of the subjects are shown in Table 1. The initial weight ($p = 0.974$) and intranasal pH values ($p = 0.204$) of subjects were similar across the groups. Throughout the experiment, all subjects survived until the termination. No surgical complications such as respiratory distress, infection, bleeding, or nasal adhesions occurred in any of the rats. The final weight ($p = 0.692$) and intranasal pH values ($p = 0.403$) of subjects were similar across the groups ($p > 0.05$). There was no significant difference between the initial and final intranasal pH values in the control and study groups ($p = 0.317$; $p = 0.564$, respectively). However, significant differences were found between the initial and final values of SW in control and study groups ($p = 0.003$; $p = 0.003$, respectively) (Table 1).

The microscopic and macroscopic outcomes of the study are presented in Table 2. In the statistical analysis of the results, the macroscopic closure rate was found to be significantly higher in the AT group ($p = 0.006$). In the evaluation of the histopathological results of the study, the count of acute inflammatory cells was significantly lower in the study group compared to the control group ($p = 0.031$). However, the fibroblast count ($p = 0.003$), vascularization ($p = 0.003$), granulation tissue amount ($p = 0.022$) and collagen density ($p = 0.044$) were significantly higher in the study group. Giant cell presence was not observed in any subject. There was no significant difference in the analysis of the other parameters ($p > 0.05$) (Table 2).

4 | Discussion

NSP, which can result from various etiologies, primarily surgical trauma, can cause to symptoms that significantly impair quality of life, such as nasal congestion, epistaxis, and inspiratory whistling [13]. Advancements in techniques and technology in endoscopic surgery, the development of various graft materials, and the identification of new pedicled local flaps have led to improvements in the outcomes of NSP repair surgery [3, 4, 14]. Nevertheless, the primary goal in managing NSP remains the prevention of perforation formation [6]. In cases where nasal septum injuries stemming from various causes fail to heal adequately, the wound may result in NSP [5, 6]. Therefore, substances known for their wound-healing properties have been studied in previous research to enhance the healing of NSP [5, 6, 15, 16]. In this study, the effects of AT plant extract on wound healing were investigated using a NSP animal model. The study has shown that AT extract reduced acute inflammation in the wound area and increased the number of fibroblast, formation of new blood vessels, granulation tissue amount, collagen density, and macroscopic closure rate of NSP.

Wound healing consists of successive, intertwined phases and this well-designed complex process concludes with the formation of scar tissue [1–4, 11]. Augmented oxidative stress, impaired nutrition, and heightened inflammation can extend this process and might also lead to chronic wound formation [5, 16, 17]. The search for a wound healing agent that ensures faster and scar-free wound healing has been the subject of numerous studies [18]. An optimal wound healing substance preserves the tissue from infection, reduces inflammation, shows antioxidant features, enhances cell proliferation, enhances new vessel formation and tissue blood flow, reduces scar formation, and shortens all phases of wound healing [1, 2, 5, 16, 17].

The wound-healing properties of various species belonging to the genus *Allium*, such as *A. sativum, A. longisepalum* Bertol, *A. stipitatum* Regel, *A. neapolitanum* Cirillo, *and A. cepa* L., have been demonstrated through numerous in vivo and in vitro studies on different tissue types [18–24]. Although the literature elucidates numerous beneficial properties of AT, an endemic species of the *Allium* genus, no prior studies have examined its effects on wound healing [8–11, 25].

The current research is designed to investigate the potential influence of the AT species on wound healing. Due to its accessibility and the ability to simultaneously examine potential effects across multiple tissue types, the nasal septum has been selected as the wound site in the current study. The perforations were formed utilizing a cannula to ensure standardization of perforation size. Wound healing is influenced by sex hormones [26]. To minimize the impact of this effect, only male subjects were included in the study. While studies exist on the oral and systemic administration of *Allium* species, there are also studies in the literature demonstrating the effectiveness of topical application in wound healing [18–24, 27]. To avoid potential systemic side effects and enhance concentration at the wound site, topical application was chosen as the route of administration in the current study. To preserve the potential synergistic effects of the constituents present in the plant, the maceration method was preferred over active ingredient extraction. Our study concluded on the 14th day to examine wound healing both macroscopically and microscopically, following a methodology consistent with existing literature [5, 16].

A critical stage of the wound healing is the inflammatory phase. In the wound area, infection increases pro-inflammatory cytokines and acute inflammation, leading to the accumulation of reactive oxygen radicals that damage blood vessels. Consequently, infection, acute inflammation, and increased oxidative stress hinder tissue regeneration and delay wound healing [28]. It has been shown that allicin compound found in *Allium* species show inhibitory effects against both Gram-positive and Gram-negative bacteria such as *Streptococcus, Staphylococcus, Escherichia, Enterobacter, Klebsiella*, and *Pseudomonas*. Additionally, the organosulfur compounds present in these species have been demonstrated to possess antiviral properties [29, 30]. Bioactive sulfur compounds such as diallyl sulfide and methyl sulfide found in *Allium* species suppress the synthesis and secretion of pro-inflammatory cytokines such as Interleukin (IL)-1 and Tumor Necrosis Factor (TNF)-alpha [23, 27, 31]. Especially allicin, and other organosulfur compounds, flavonoids, phenolic compounds, and elements such as selenium, demonstrate robust antioxidant properties by inhibiting enzymes responsible for reactive oxygen species (ROS) production and directly binding to ROS [23, 27–29, 32]. The observed decrease in acute inflammation in the AT group in this study may be attributed to the

TABLE 2 | The investigation of the histopathological parameters and macroscopic closure rates among the groups.

	Groups ($n=22$)										
	Study ($n=11$) n (%)					Control ($n=11$) n (%)					
Parameters	**None**	**Mild**	**Moderate**	**Intensive**	**Median(Q1–Q3)**	**None**	**Mild**	**Moderate**	**Intensive**	**Median(Q1–Q3)**	***p***
Acute inflammatory cells count	8 (72.7)	3 (27.3)	0 (0)	0 (0)	0 (0–1)	3 (27.3)	7 (63.6)	1 (9.1)	0 (0)	1 (0–1)	**0.031***
Fibroblast count	0 (0)	0 (0)	3 (27.3)	8 (72.3)	3 (2–3)	0 (0)	0 (0)	9 (81.8)	1 (9.1)	2 (2–2)	**0.003***
Collagen density	0 (0)	0 (0)	6 (54.5)	5 (45.5)	2 (2–3)	0 (0)	1 (9.1)	9 (81.8)	1 (9.1)	2 (2–2)	**0.044***
Vascularization	0 (0)	0 (0)	4 (36.4)	7 (63.6)	3 (2–3)	0 (0)	5 (45.5)	5 (45.5)	1 (9.1)	2 (1–2)	**0.003***
Granulation tissue	0 (0)	1 (9.1)	6 (54.5)	4 (36.4)	2 (2–3)	0 (0)	6 (54.5)	4 (36.4)	1 (9.1)	1 (1–2)	**0.022***
Epithelial regeneration	0 (0)	0 (0)	3 (27.3)	8 (72.7)	2 (2–3)	0 (0)	0 (0)	7 (63.6)	4 (36.4)	3 (2–3)	0.094
Epithelial degeneration	9 (81.8)	2 (18.2)	0 (0)	0 (0)	0 (0–0)	7 (63.6)	4 (36.4)	0 (0)	0 (0)	0 (0–1)	0.35
Cartilage regeneration	0 (0)	1 (9.1)	4 (36.4)	6 (54.5)	3 (2–3)	0 (0)	2 (18.2)	7 (63.6)	2 (18.2)	2 (2–2)	0.104
Cartilage degeneration	8 (71.7)	3 (27.3)	0 (0)	0 (0)	0 (0–1)	6 (54.5)	5 (45.4)	0 (0)	0 (0)	0 (0–1)	0.386
Eosinophil count	5 (45.5)	4 (36.4)	2 (18.2)	0 (0)	1 (0–1)	8 (71.7)	2 (18.2)	1 (9.1)	0 (0)	0 (0–1)	0.218
Macroscopic perforation size	Enlarged	Unchanged	Reduced	Closed		Enlarged	Unchanged	Reduced	Closed		
	0 (0)	0 (0)	0 (0)	11 (100)	3 (3–3)	0 (0)	3 (27.3)	3 (27.3)	5 (45.5)	3 (2–3)	**0.006***

*The Mann–Whitney U test, $p<0.05$.

known antioxidant properties and potential anti-inflammatory and antibacterial effects of AT species.

Previous studies have demonstrated that the *Allium* genus increases the number of fibroblast, key cell in wound healing, and increases the production and maturation of collagen, the primary component in the remodeling stage, that is the most crucial stage of wound healing [22, 27, 31]. Previous studies have demonstrated that species belonging to the *Allium* genus accelerate epithelialization, one of the earliest components of wound healing, and enhance the formation of new blood vessels, which are crucial for supplying the necessary nutrients for all phases of the wound healing process [19–21, 33]. It is an anticipated phenomenon that species of the *Allium* genus, which increase the components of granulation tissue, also enhance the amount of granulation tissue formed [22]. In the current study, the observed increase in fibroblast and new blood vessel counts, as well as the augmented formation of granulation tissue and collagen in the AT group, may be attributed to the effects of the *Allium* genus on the proliferative and remodeling phase.

In the study conducted by Dietz [34], the intranasal pH in rats was determined to be between 7.25 and 7.30. In this study, the determined intranasal pH values were consistent with these values; however, no significant pH change was detected due to the use of AT. Additionally, weight gain, indicative of welfare status in experimental animals, was observed in the subjects [35]. Furthermore, the absence of a statistically significant difference in eosinophil count, an indicator of allergic reactions, between the treatment and control groups, as well as the lack of giant cells, a microscopic indicator of foreign body reaction, may be interpreted as evidence that the topical application of AT does not induce adverse effects in the organism [5, 6].

This experimental study, which is the first in the literature to reveal the beneficial influence of AT on wound healing, has certain limitations. The primary limitation is that the histopathological evaluation, which forms the foundation of the current outcomes, was conducted using a subjective scoring system. To mitigate this effect, the histopathological evaluations were performed by a histopathologist with a decade of experience at a tertiary healthcare center. The value of the obtained results is limited by the lack of significant differences in the other examined parameters, notably the observed increase in epithelial regeneration. This could be attributed to the fact that wound healing was not yet complete on the 14th day, the end day, and to the small sample size determined in accordance with the ethical "reduction principle." The study termination day was consistent with existing studies in the literature. The sample size was determined at the beginning of the study using a statistical program, and the obtained results are statistically robust.

5 | Conclusion

The current experimental study demonstrated that topical *A. tuncelianum* solution may enhance wound healing in a NSP rat model. *A. tuncelianum* may have the potential to be utilized topically as an effective therapeutic agent to prevent permanent NSP when applied during the wound healing period. The reiteration of our findings through more exhaustive studies, involving a larger sample size, multiple dosage intervals, and objective examination methods, is necessary to substantiate the results.

Conflicts of Interest

The authors declare no conflicts of interest.

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