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ARTICLE

The effect of Sambucus nigra extract in the treatment of interstitial cystitis

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Objective: *Sambucus nigra (SN) has been found to exhibit strong antioxidant properties and anti-inflammatory effects. In our study, we aimed to investigate the therapeutic effects of Sambucus nigra extracts (SNe) in interstitial cystitis, a condition in which inflammation plays a significant role in its pathophysiology.*

Methods: *Thirty Wistar albino adult female rats were used in this study. All rats were housed at an average room temperature of 23°C, with a 12-h light/dark cycle, and had ad libitum access to food. The rats were divided into three groups: Group 1 (n = 10): Control (sham) group, Group 2 (n = 10): Interstitial cystitis group, and Group 3 (n = 10): Treatment group. Rats in Group 3 were administered oral Sambucus nigra extract at a dose of 0.040 g/kg every other day for a total of 8 weeks. Bladder tissues were examined both histologically and immunohistochemically, with a focus on mast cells and the presence of Interleukin-6 (IL-6), Interleukin-8 (IL-8), Vascular Endothelial Growth Factor (VEGF), and Tumor Necrosis Factor-α (TNF-α).*

Results: *The findings of chronic inflammation were striking in the bladder sections of the interstitial cystitis group. In the treatment group, regenerating transitional epithelium, a compact appearance in the lamina propria, and a decrease in inflammatory cells and mast cells were observed. While significant improvements were noted in IL-8, TNF-α, and mast cell counts, there was a reduction in IL-6 and VEGF levels, although this reduction was not statistically significant.*

Conclusion: *Sambucus nigra, significantly reduces TNF-α, Mast cell, VEGF, IL-6, and IL-8 levels in interstitial cystitis (IC) bladder tissue. It provides recovery in damaged bladder epithelium, smooth muscle, and basal membrane. SN attracts attention as an important agent due to its effects in the treatment of IC.*

Key Words: interstitial cystitis, *Sambucus nigra*, inflammation, interleukin-8, tumor necrosis factor-α

Introduction

Interstitial cystitis (IC), a chronic disease characterized by lower urinary tract symptoms and suprapubic pain, with an etiology that has not been fully elucidated, is often reported as painful bladder syndrome (IC/BPS) in some cases.[1] Additionally, IC is commonly associated with urinary urgency.[2] The variability in diagnostic criteria, differences in community characteristics, and study design complicate epidemiological data. Nevertheless, its incidence is 21–100 per 100,000, and it is observed in women at a rate 5 times higher than in men.[3] The prevalence of IC in the literature ranges from 0.01% to 6.5%.[4]

There are many studies in the literature regarding the etiology and pathophysiology of interstitial cystitis. Although its pathophysiology has not been fully elucidated, epithelial dysfunction, mast cell activation, autoimmunity, and an increase in C-type nerve fibers in the bladder play a role in its pathogenesis.[3,5] Research in the literature has shown that inflammation plays a key role in the etiology of interstitial cystitis.[6] The neuroinflammatory response mediated by various mediators is

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thought to increase epithelial permeability, leading to fibrosis, excessive detrusor activity, and a reduction in compliance, ultimately resulting in a progressive process.[7] In pathological diagnosis, subepithelial infiltration of polymorphonuclear leukocytes, lymphocytes, mast cells, plasma cells, and the presence of fibrotic lesions are investigated.[8] Studies have shown that inflammatory cytokines play a significant role in the pathogenesis of IC and the development of its symptoms. Levels of inflammatory cytokines such as interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), tumor necrosis factor-α (TNF-α), and vascular endothelial growth factor (VEGF) are significantly elevated in IC patients.[9,10] Treatments using anti-inflammatory and antioxidant extracts have been shown to decrease mast cell numbers in the bladder and reduce levels of inflammatory cytokines like IL-6, IL-8, and TNF-α in experimental IC rat models.[2,10,11] This suggests that anti-inflammatory treatment strategies may have a potential role in patients with interstitial cystitis.

Sambucus (Elderberry) is a short-statured plant belonging to the Adoxaceae family. While it has several subspecies, Black Elder (Elder Flower, *Sambucus nigra* L.) and Dwarf Elder (*Sambucus Ebulus* L.) are used for therapeutic purposes in some cultures.[12] In this regard, *Sambucus nigra* is a plant that holds a place in traditional medicine due to its antioxidant and diuretic properties. It has been shown to exhibit strong antioxidant properties[13] and to inhibit the biosynthesis of inflammatory cytokine TNF-α.[14] Similarly, extracts of Sambucus Ebulus have been found to demonstrate anti-nociceptive and anti-inflammatory effects.[15]

In our study, we aimed to investigate the therapeutic effects of Sambucus extracts in interstitial cystitis, a condition in which inflammation plays a significant role in its pathophysiology.

Materials and Methods

Study design

This study used 30 conventional, non–specific pathogen-free (non-SPF) adult healthy female Wistar albino rats, aged 10–12 weeks and weighing 200–250 g, bred in accordance with FELASA (Federation of European Laboratory Animal Science Associations) standards. Rats were obtained from Aydin Adnan Menderes University, Faculty of Medicine, Experimental Animal Production and Experimental Research Laboratory. When determining the total number of animals in the study groups, the sample size was calculated using the "resource equation" method.[16] It was planned to have a maximum of 10 rats in groups, with 21 degrees of freedom. All rats were followed up at an average room temperature of 23°C, in a 12-h dark-light cycle, and on an ad libitum diet. Rats were divided into 3 groups: Group 1 (n = 10): Control (sham) group, Group 2 (n = 10): Interstitial cystitis group and Group 3 (n = 10): Treatment group. Ethics committee approval was obtained from Aydın Adnan Menderes University Animal Ethics Committee (No.: 64583101/2021/015).

Formation of groups

Rats in Groups 2 and 3 were administered intraperitoneal 75 mg/kg cyclophosphamide (Endoxan 1 g injection, Eczacibasi Baxter, Istanbul, Türkiye) every 3 days for 4 doses, as previously described in the literature.[2] Control group rats received an intraperitoneal injection of serum physiologic (0.9% NaCl) at a similar volume. After a 12-day preparation period, rats in Group 3 were started on oral administration of *Sambucus nigra* extract (0.04 g/kg). The extract used for treatment was prepared from the precipitate obtained after processing dried *S. nigra* fruit with methanol (NPRO Natural Product Biotechnology, Izmir, Türkiye). The phenolic content of the extract was measured and expressed as gallic acid equivalent (GAE/100 mg extract). The prepared extract was mixed in distilled water and dimethyl sulfoxide (DMSO) to create a solution that was administered orally to rats (95 mL distilled water + 5 mL DMSO). The daily dose administered to the rats was determined to be 0.040 g/kg, based on similar studies in the literature.[17,18] The treatment dose was determined based on the recommended lethal dose 50% (LD_{50}) doses for the extract.[15,19] The amount decided as the final treatment dose was based on the half-maximal inhibitory concentration (IC_{50}) dose at which the extract exhibited maximal antioxidant effect.[13] The phenolic content of the extract has been demonstrated in previous studies[20] and the extract was prepared by standardizing it as shown in previous studies.[21] The treatment was applied every other day as part of the daily diet for a total of 8 weeks. At the end of the treatment, all rats in the groups underwent intraperitoneal ketamine (90 mg/kg)/xylazine (10 mg/kg) anesthesia, laparotomy was performed, and bladder tissues were excised and fixed in 10% formalin.

Hematoxylin-eosin staining and may grunwald giemsa staining method

Sections taken from all groups were kept in an oven. They were removed from xylol and cleared of paraffin.

The sections were stained at room temperature (approximately 20°C–25°C and the relative humidity is between 35%–40%) with 1% hematoxylin prepared in a pH 3.0 medium for 4–5 min and rinsed with phosphate-buffered water at pH 6.8. Subsequently, counterstaining was performed with 1% eosin prepared in a pH 4.5 medium for 1–1.5 min to complete the staining procedure. For the evaluation of mast cells, other preparations were stained with 1% May–Grünwald solution for 3–5 min and briefly rinsed with phosphate buffer at pH 6.8, followed by staining with 5% Giemsa solution prepared in the same buffer for 10 min. The slides were then rinsed briefly with the buffer and prepared for microscopic evaluation. For the microscopic evaluation, an Olympus BX51 microscope manufactured by Olympus Corporation, located at 2951 Ishikawa-machi, Hachioji, Tokyo 192-8507, Japan, was used.

Immunohistochemical staining method

Sequenza Immunostaining Center Each73300001 Shandon/ThermoScientific IHC device used for staining (Thermo Scientific, Waltham, MA, USA). 3 µm-thick sections taken from bladder tissue paraffin blocks were applied with a 1/10 diluted Citrate Buffer (pH: 6) (AP-9003-999 Thermo Scientific) PT Module (A80400012 LabVision) to unmask the antigen, Protein Blocked (TA-125-PBQ Thermo Scientific) for 10 min. IL8 antibodies (orb229133, 1/50, Biorbyt, Cambridge, UK), IL-6 antibodies (orb539985, Biorbyt, 1/100), VEGF antibodies (orb191500, Biorbyt, 1/50), and TNF alpha antibodies (orb11495 Biorbyt, 1/100), and washing was done with PBS (concentration 0.5×, pH 7.4). DAB Chromogen (TA-125-HA, Thermo Scientific) is used to identify positive cells.

Immunohistochemical and mast cell count evaluation

May-Grunwald Giemsa stain was performed to evaluate mast cells. While determining the number of mast cells in the groups, the average of the number of mast cells counted from 5 randomly selected fields at ×100 magnification was used. Cytoplasmic staining was investigated immunohistochemically. In all groups, 100 cells randomly selected from the strongest areas were counted to calculate the percentage of IL-6, IL-8, VEGF and TNFα.

Immunohistochemical staining results were evaluated as follows: (+++): strong staining, (++): moderate staining, (+): weak staining, (/+/−): very weak staining, (−): no staining.

Histological and immunohistochemical evaluations were made by an expert pathologist.

Statistical analyses

All results were expressed as means ± standard deviation (SD). Significant values in the groups were assessed with one-way ANOVA. Statistical analyses were calculated using GraphPad Prism version 7.04 for Windows (GraphPad Software, San Diego, CA, USA). $p < 0.05$ was considered significant. IHC staining results in groups were evaluated with the Kruskal-Wallis and Bonferroni post-hoc test using SPSS software version 22 (SPSS Inc., Chicago, IL, USA). $p < 0.001$ was considered significant.

Results

In the IC group, chronic inflammation is observed in the transitional epithelium and lamina propria with H&E staining. In the control group, intact transitional epithelium and an intact basement membrane are observed in the bladder tissue. In the treatment group, regenerating transitional epithelium, a compact appearance in the lamina propria, and a decrease in inflammatory cells are observed (Figure 1). A significant decrease in mast cell count was also noted (Figure 2).

In group 2 rats with the IC model, immunohistochemical staining showed significant increases in IL-6, IL-8, TNF-α, and VEGF levels compared to the control group (Table 1). In rats treated with *Sambucus nigra* extract, the levels of IL-6, IL-8, TNF-α, and VEGF were lower compared to the IC-induced rats (Figures 3–6). This decrease was statistically significant for IL-8 and TNF-α ($p = 0.045$ and $p = 0.003$), but not for IL-6 and VEGF ($p = 0.183$ and $p = 0.465$) (Table 1 and Figure 7).

Discussion

The International Continence Society (ICS) defines interstitial cystitis (IC) as a chronic condition characterized by persistent or recurrent chronic pelvic pain, pressure, or discomfort, accompanied by at least one urinary symptom such as urgency or frequent urination, without any pathology to explain the findings.[22] These symptoms significantly reduce the quality of life. In addition to being a major handicap in the social life of patients, IC also affects their working hours and job performance, creating an economic burden. The annual healthcare costs of IC/BPS are 2.0 to 2.4 times higher than those for healthy people of the same age.[3] Therefore, IC is one of the significant conditions in urology

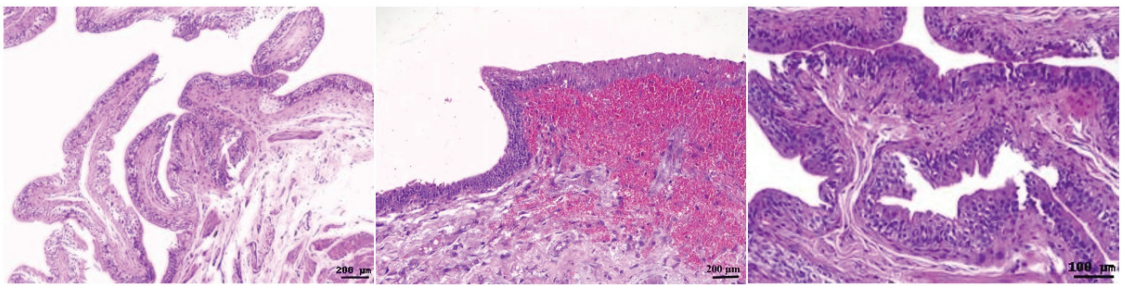

FIGURE 1. Hematoxylin-Eosin staining results in groups. (**A**) In the control group, intact transitional epithelium and intact basement membrane are observed in the bladder tissue (H&E, ×5). (**B**) In the IC group, chronic inflammation is observed in the transitional epithelium and lamina propria, (H&E, ×5). (**C**) In the treatment group, regenerating transitional epithelium, a compact appearance in the lamina propria and a decrease in inflammatory cells are observed, (H&E, ×10). IC, Interstitial cystitis

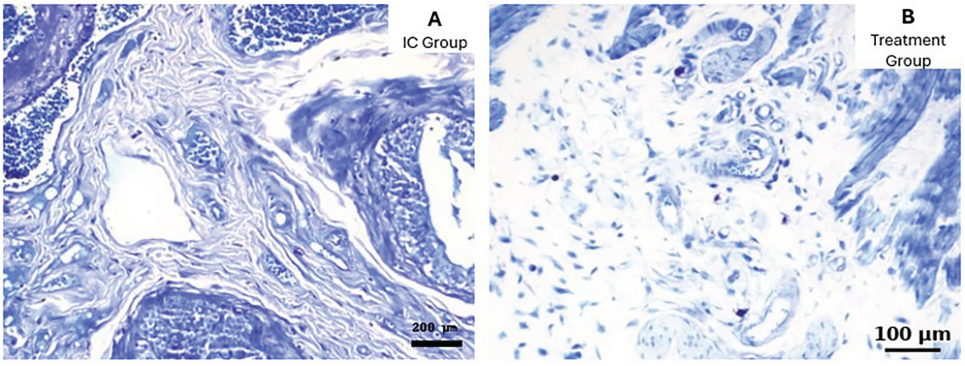

FIGURE 2. Mast cells with Giemsa staining. (**A**) Section of Group 2 rats (IC model, ×5), (**B**) Section of Group 3 rats (IC model with treatment of SNe, ×10). Mast cell count was observed to be significantly lower in the treatment group rats (See also Figure 7 for quantification). IC, Interstitial cystitis; SNe, *Sambucus nigra* extract

practice. There are many treatment options, including behavioral/non-pharmacological therapies, oral medications, bladder instillations, or surgery. The therapeutic goal is to relieve bladder pain, reduce urgency and frequency, and improve patients' quality of life (QoL) (3). Although oral and intravesical treatments can control symptoms in patients with mild to moderate symptoms, the relapse rates remain high. As a result, the search for effective treatments continues.[23] In addition, recent studies have shown that the urinary microbial ecosystem, called urobiome, plays an important role in inflammatory urinary system diseases such as interstitial cystitis, urethritis, and chronic prostatitis.[24] Disruption of the urobiome equilibrium leads to excessive proliferation of pathogenic microorganisms, the development of inflammatory urinary tract diseases and triggers the activation of the immune system. Repairing urinary microbiota with probiotic therapies plays an important role in reducing inflammation and preventing infections.[24] In this regard, our findings support that *S. nigra* extracts show promising potential in the treatment of IC. And to our knowledge, this is the first study conducted with SNe in this area.

S. nigra is used in the market as a food supplement in many countries for its medical benefits as well as traditional medical applications. It shows anti-inflammatory, antioxidant, antitumor and immunomodulatory activities with the bioactive substances such as polyphenols and flavonoids it contains.[14,19] Experimental studies show that sambucus extracts are potent hydroxyl radical scavengers. Similarly, it has been found to inhibit lipid peroxidation and have an antioxidant effect.[13] It has also been shown to inhibit nitric oxide

TABLE 1. **Immunohistochemical staining rates between groups**

IHC and Giemsa Staining	Group 1 (Control)	Group 2 (IC model)	Group 3 (IC model + SNe Treatment)	*p (ANOVA)*	*p (Post-HOC)*
IL-6	4.50[a,c]	18.50[a,b,c]	14.5[a,b]	<0.001[a]	0.183[b] <0.001[c]
IL-8	5.44[a,c]	20.06[a,b,c]	12.00[a,b]	<0.001[a]	0.045[b] 0.028[c]
TNF-α	4.50[a,c]	20.19[a,b,c]	12.81[a,b]	<0.001[a]	0.003[b] <0.001[c]
VEGF	5.38[a,c]	17.25[a,b,c]	14.88[a,b]	0.002[a]	0.465[b] 0.006[c]
Mast Cell Count	14.06[a,c]	17.69[a,b,c]	5.75[a,b]	0.002[a]	0.003[b] 0.375[c]

Note: IC, Interstitial Cystitis; SNe, *Sambucus nigra* Extract; IHC, Immunohistochemical Staining. [a]Analysis result between all groups; [b]Analysis result between the IC group and the treatment group; [c]Analysis result between the IC group and the control group.

synthesis in a dose-dependent manner.[13,25] Tumor necrosis factor, plays a key role in inflammation and the immune response and *S. nigra* inhibits TNF-α biosynthesis. Thus, it may have anti-inflammatory and antirheumatic effects.[14] Schwaiger et al. showed that ursolic acid, one of the active components of sambucus extract, inhibited TNF-α-induced expression of vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecule 1 (ICAM-1). Increased VCAM1 expression is associated with several chronic inflammatory diseases, which may make it a target for therapy.[26] Similarly, ursolic acid inhibits cyclooxygenase-2 (COX2) gene expression and prostaglandin (PG) synthesis, confirming that it can have both anti-inflammatory and chemopreventive effects.[27] Nuclear factor erythroid 2-related factor 2 (Nrf2) is a key component of endogenous antioxidant defense systems that regulates mitochondrial ROS production. Nrf2 induces the heme oxygenase-1 (HO-1) gene by increasing mRNA and protein expression. HO-1 expression suppresses nuclear factor-κB (NF-κB) activation, leading to inhibited secretion of inflammatory cytokines. NF-κB activation also stimulates the protein release of VEGF to induce angiogenesis.[28] Lin et al. showed that sambucus extracts suppressed NF-κB protein expression. And it was shown to downregulate the levels of IL-6 and VEGF in a dose-dependent manner.[29] Current

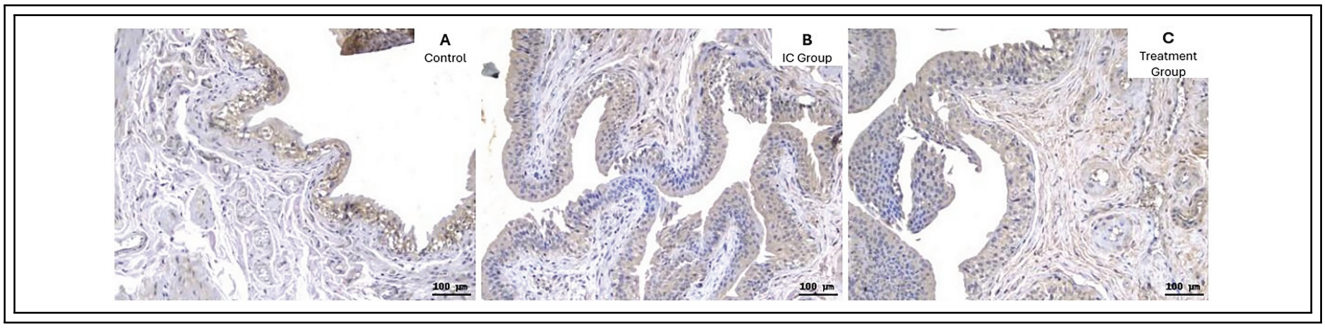

FIGURE 3. Immunohistochemical stainings with IL-6 between the groups. (**A**) Control group with IL-6 staining, 0–25% (×10), (**B**) IC group with IL6 staining, 75–100% (×10), (**C**) Treatment group with IL-6 staining, 75–100% (×10). Immunohistochemical staining showed that IL6 levels were decreased in SNe-treated rats. However, this difference was not statistically significant (See also Figure 7 for quantification). IC, Interstitial cystitis; IL-6, Interleukin-6

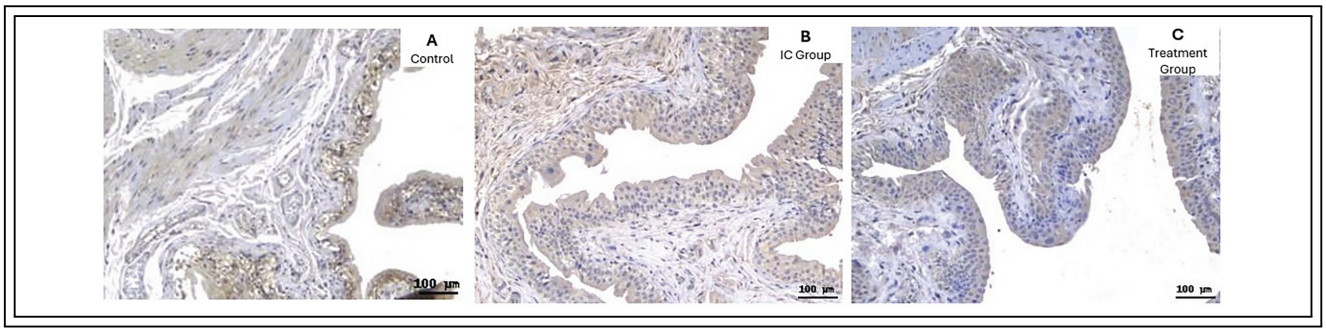

FIGURE 4. Immunohistochemical stainings with IL-8 between the groups. (**A**) Control group with IL-8 staining, 25–50% (×10), (**B**) IC group with IL8 staining, 75–100% (×10), (**C**) Treatment group with IL-8 staining, 50–75% (×10). Immunohistochemical staining showed that IL8 levels were significantly decreased in SNe-treated rats (See also Figure 7 for quantification). IC, Interstitial cystitis; IL-8, Interleukin-8

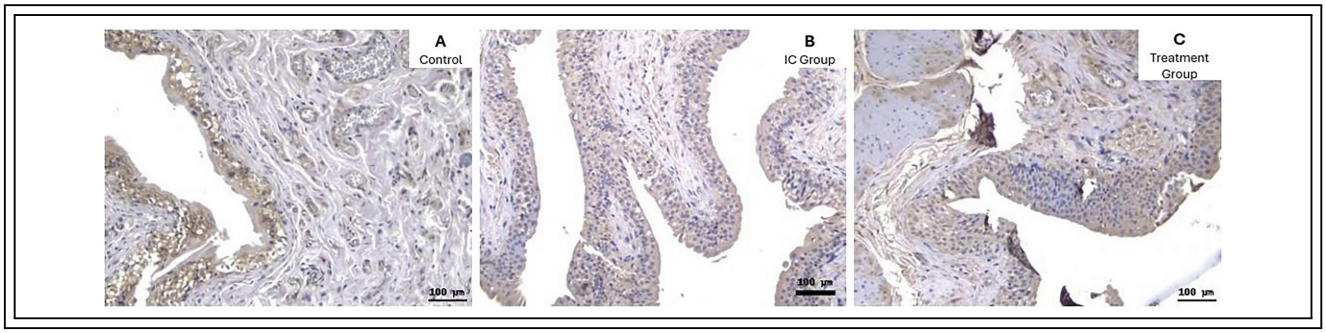

FIGURE 5. Immunohistochemical stainings with TNFα between the groups. (**A**) Control group with TNFα expression, 25–50% (×10), (**B**) IC group with TNFα expression, 75–100% (×10), (**C**) Treatment group with TNFα expression, 50–75% (×10). Immunohistochemical staining showed that TNFα levels were significantly decreased in SNe-treated rats (See also Figure 7 for quantification). IC, Interstitial cystitis; TNFα, Tumor necrosis factor-alpha

findings suggest that Sambucus extracts may have a role in the treatment of IC.

The importance of mast cell infiltration in IC/BPS is frequently reported in studies, although its significance remains unclear. In interstitial cystitis patients, there is an increase in mast cells in both the lamina propria and detrusor muscle of the bladder tissue.[30] Studies have shown that detrusor mastocytosis is associated with fibrosis and may indicate the need for further treatment.[7] Similarly, the increase in mast cells has been observed to correlate with lymphoplasmacytic cell infiltration in the subepithelial tissue.[31] Mast cell activation leads to the release of various mediators such as histamine, kinins, proteases, IL-6, IL-8, and nitric oxide, which are inflammatory, nociceptive, and vasoactive.[6] These mediators contribute to neuroinflammatory responses, which increase epithelial permeability, leading to the development of a chronic process characterized by fibrosis, detrusor hyperactivity, and reduced compliance.[7] In human studies, a histological number of $\geq$28 cells/mm^2 has been used to diagnose IC/BPS,[32] but no such cut-off value has been reported for rat studies. In our study, where mast cells were quantitatively assessed, we observed an increase in mast cell count in the bladder tissue of rats with interstitial cystitis compared to the control group. However, in rats treated with *Sambucus nigra* extract, we found a statistically significant reduction in mast cell count (Mean 17.69 vs. 5.75; p: 0.003). This finding supports the potential therapeutic benefit of *Sambucus nigra* extracts in the treatment of interstitial cystitis.

Inflammatory cytokines such as IL-6, IL-8, and TNF-α are significantly elevated in patients with IC.[9] IL-6 is secreted by inflammatory cells, including macrophages and mast cells. Studies have shown that serum and urinary IL-6 levels are elevated

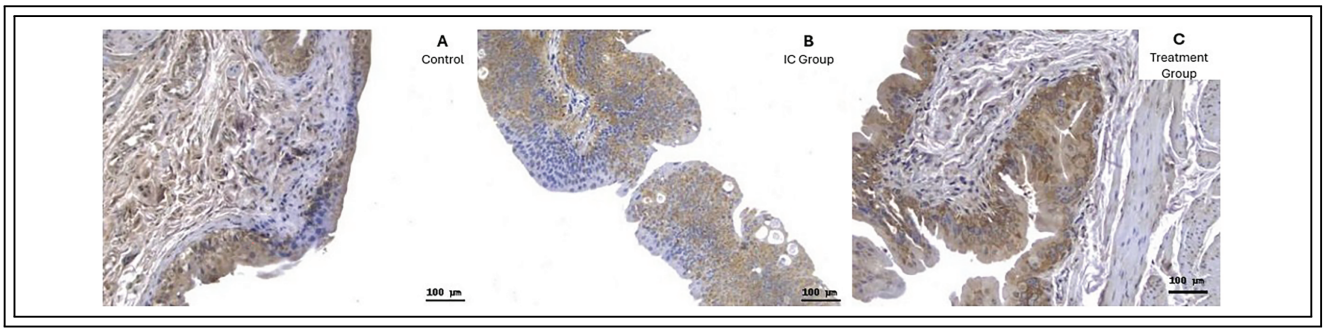

FIGURE 6. Immunohistochemical stainings with VEGF between the groups. (**A**) Control group with VEGF expression, 25–50% (×10), (**B**) IC group with VEGF expression, 75–100% (×10), (**C**) Treatment group with VEGF expression, 50–75% (×10). Immunohistochemical staining showed that VEGF levels were decreased in SNE-treated rats. However, this difference was not statistically significant. IC, Interstitial cystitis; VEGF, Vascular endothelial growth factor

in IC patients, and these levels correlate with symptom severity.[33] IL-6 levels may trigger afferent sensitization, and it has been reported that they may contribute to bladder pain symptoms.[34] A study investigating urinary chemokines in ulcerative IC/BPS reported that IL-6 levels are significantly increased up to 20-fold compared to non-ulcerative IC/BPS patients.[35] IL-8, a chemotactic cytokine, increases the inflammatory response through the migration of neutrophils, eosinophils, and T lymphocytes.[2] It also plays a critical role in normal urothelial growth and its continuity.[36] While it has been shown that IL-8 levels could be a high-sensitivity biomarker for diagnosing IC, no significant relationship with the clinical features of the disease has been found.[37] Jiang et al. found that urinary IL-8 levels are significantly elevated in IC/BPS patients in their study.[9] An older study reported a strong positive relationship between urinary IL-8 levels and bladder mast cell counts.[38] Similarly, our results showed that in rats with IC, IL-6 and IL-8 levels were significantly higher compared to the control group. Additionally, inflammation was observed in the transitional epithelium and lamina propria of the bladder mucosa in these rats. In rats treated with SNe, IL-6 and IL-8 levels were reduced. While the reduction in IL-8 levels was statistically significant, the decrease in IL-6 levels was not statistically significant. However, H&E staining revealed that in the treatment group, the inflammatory response in the bladder mucosa was significantly reduced.

TNF-α is released from activated macrophages and leads to an increase in reactive oxygen species (ROS) and the expression of adhesion molecules on the vascular endothelium. It also serves as a chemotactic agent for monocytes and polymorphonuclear leukocytes, thereby contributing to the inflammatory response.[39] Similarly, in IC, inflammation mediated by TNF-α and IL-6, as well as the production of free oxygen radicals, has been associated with urothelial damage. IL-6 is also highlighted for increasing detrusor contractility, leading to the onset of urinary symptoms.[11] Another important pathological feature in IC/BPS is bladder fibrosis.[7] Jin et al. stimulated epithelial cells with TNF-α in an *in vitro* study using human uroepithelial cells, and as a result, observed collagen accumulation leading to pro-fibrogenesis due to TNF-induced inflammation.[40] Similarly, levels of VEGF, a marker of angiogenesis, are elevated in patients with IC.[10] It has also been reported that VEGF may serve as a useful biomarker for the diagnosis of BPS/IC.[35] VEGF may contribute to the hyperalgesia experienced by IC/BPS patients and is potentially linked with neuronal control and nerve repair in these patients.[41] VEGF expression in IC patients is thought to be caused by hypoxia in bladder tissues during the filling phase. This increased VEGF expression is thought to contribute to bladder fibrosis and a decrease in bladder capacity.[42] VEGF is associated with angiogenesis, and its inhibition leads to a reduction in inflammation.[43] Anti-angiogenic therapy may be considered as a treatment method in IC. Similarly, intravesical administration of platelet-rich plasma has been shown to reduce VEGF levels and help repair the urothelium.[44] Our results demonstrate that treatments with *Sambucus nigra* extract may reduce TNF-α and VEGF levels. In treated rats, this improvement was statistically significant for TNF-α when compared to the IC group (mean 20.19 vs. 12.81, $p = 0.003$), but for VEGF, the reduction was not statistically significant (mean 17.25 vs. 14.88, $p =$

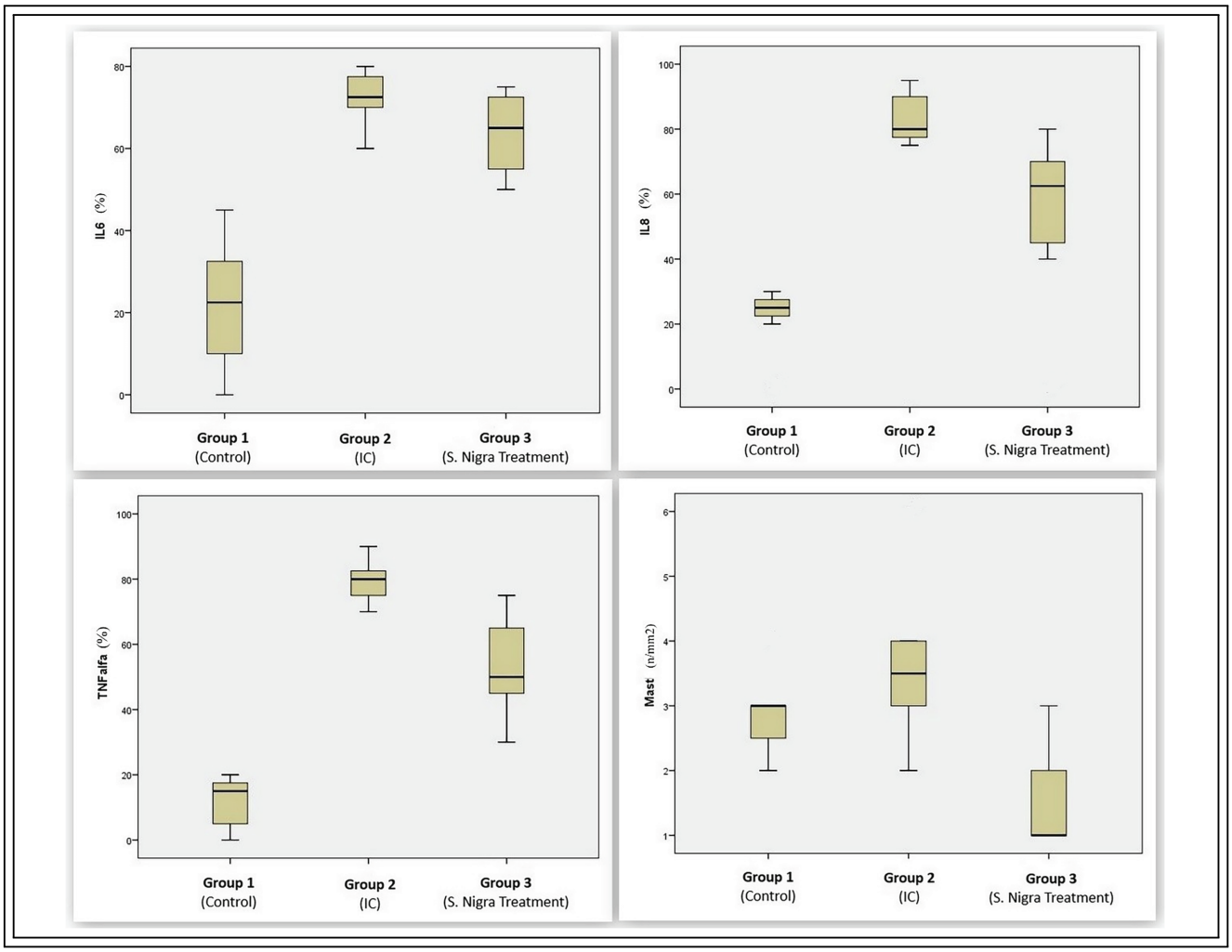

FIGURE 7. Immunohistochemical and Giemsa staining results. Decreased levels of IL-6, IL-8 and TNF-α, as well as reduced mast cell numbers, were shown in rats treated with *S. nigra* extract. Group 1, The control group; Group 2, Rats of the interstitial cystitis model; Group 3, Rats treated with SNe

0.465). In correlation with these results, H&E staining showed that in the treated rats, regenerating transitional epithelium, a compact appearance in the lamina propria, and a decrease in inflammatory cells were observed. This is in line with the literature[44] and supports the idea that *S. nigra* extracts may have therapeutic potential in the treatment of IC.

Being an experimental study and lack of urodynamic evaluations are the shortcomings of this study. Nevertheless, we believe that our findings will guide randomized, prospective studies that also evaluate quality of life indices.

The results of our study support the potential of *S. nigra* extract, which holds a place in traditional medicine, as a promising treatment for IC patients. We believe that clinical studies supporting our findings will pave the way for further therapeutic approaches in this field. Additional studies are needed to determine a standardized effective and safe dose for oral use or intravesical use in patients with interstitial cystitis.

Conclusion

Sambucus nigra, significantly reduces TNF alpha, Mast cell, VEGF, IL-6 and IL-8 levels in IC bladder tissue. When applied as treatment, it provides recovery in damaged bladder epithelium, smooth muscle and basal membrane. For this reason, SN attracts attention as an important agent to investigate its effects in the treatment of IC.

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Author Contributions

Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Visualization, Writing—Original Draft Preparation, Writing—Review & Editing: Arif Kol; Conceptualization, Data Curation, Funding Acquisition, Investigation, Methodology, Resources, Supervision, Visualization, Writing—Original Draft Preparation: Hüseyin Günizi; Conceptualization, Data Curation, Funding Acquisition, Investigation, Methodology, Resources, Validation, Writing—Original Draft Preparation: Özlem Ceren Günizi. All authors reviewed the results and approved the final version of the manuscript.

Availability of Data and Materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics Approval

The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the Ethical Review Board of the Aydın Adnan Menderes University Animal Ethics Committee (No: 64583101/2021/015).

Informed Consent

Not applicable.

Conflicts of Interest

The authors declare no conflicts of interest to report regarding the present study.

References

1. Jones CA, Nyberg L. Epidemiology of interstitial cystitis. *Urology* 1997;49(5A Suppl):2–9. doi:10.1016/s0090-4295(99)80327-6.
2. Li W, Yang F, Zhan H et al. *Houttuynia cordata* extract ameliorates bladder damage and improves bladder symptoms via anti-inflammatory effect in rats with interstitial cystitis. *Evid Based Complement Alternat Med* 2020;2020(1):9026901. doi:10.1155/2020/9026901.
3. Li J, Yi X, Ai J. Broaden horizons: the advancement of interstitial cystitis/bladder pain syndrome. *Int J Mol Sci* 2022;23(23):14594. doi:10.3390/ijms232314594.
4. Homma Y, Akiyama Y, Tomoe H et al. Clinical guidelines for interstitial cystitis/bladder pain syndrome. *Int J Urol* 2020;27(7):578–589. doi:10.1111/iju.14234.
5. Sant GR. Etiology, pathogenesis, and diagnosis of interstitial cystitis. *Rev Urol* 2002;4(Suppl 1):S9.
6. Grover S, Srivastava A, Lee R, Tewari AK, Te AE. Role of inflammation in bladder function and interstitial cystitis. *Ther Adv Urol* 2011;3(1):19–33. doi:10.1177/1756287211398255.
7. Richter B, Hesse U, Hansen AB, Horn T, Mortensen SO, Nordling J. Bladder pain syndrome/interstitial cystitis in a Danish population: a study using the 2008 criteria of the European Society for the Study of Interstitial Cystitis. *BJU Int* 2010;105(5):660–667. doi:10.1111/j.1464-410X.2009.08847.x.
8. van de Merwe JP, Nordling J, Bouchelouche P et al. Diagnostic criteria, classification, and nomenclature for painful bladder syndrome/interstitial cystitis: an ESSIC proposal. *Eur Urol* 2008;53(1):60–67. doi:10.1016/j.eururo.2007.09.019.
9. Jiang YH, Peng CH, Liu HT, Kuo HC. Increased pro-inflammatory cytokines, C-reactive protein and nerve growth factor expressions in serum of patients with interstitial cystitis/bladder pain syndrome. *PLoS One* 2013;8(10):e76779. doi:10.1371/journal.pone.0076779.
10. Furuta A, Suzuki Y, Igarashi T et al. Angiogenesis in bladder tissues is strongly correlated with urinary frequency and bladder pain in patients with interstitial cystitis/bladder pain syndrome. *Int J Urol* 2019;26(Suppl 1):35–40. doi:10.1111/iju.13972.
11. He YQ, Zhang WT, Shi CH, Wang FM, Tian XJ, Ma LL. Phloroglucinol protects the urinary bladder via inhibition of oxidative stress and inflammation in a rat model of cyclophosphamide-induced interstitial cystitis. *Chin Med J* 2015;128(7):956–962. doi:10.4103/0366-6999.154316.
12. Młynarczyk K, Walkowiak-Tomczak D, Łysiak GP. Bioactive properties of *Sambucus nigra* L. as a functional ingredient for food and pharmaceutical industry. *J Funct Foods* 2018;40:377–390. doi:10.1016/j.jff.2017.11.025.
13. Stoilova I, Wilker M, Stoyanova A, Krastanov A, Stanchev V. Antioxidant activity of extract from elder flower (*Sambucus nigra* L.). *Herba Pol* 2007;53(1):45–54.
14. Yeşilada E, Ustün O, Sezik E, Takaishi Y, Ono Y, Honda G. Inhibitory effects of Turkish folk remedies on inflammatory cytokines: interleukin-1alpha, interleukin-1beta and tumor necrosis factor alpha. *J Ethnopharmacol* 1997;58(1):59–73. doi:10.1016/s0378-8741(97)00076-7.

15. Ahmadiani A, Fereidoni M, Semnanian S, Kamalinejad M, Saremi S. Antinociceptive and anti-inflammatory effects of *Sambucus ebulus* rhizome extract in rats. *J Ethnopharmacol* 1998;61(3):229–235. doi:10.1016/s0378-8741(98)00043-9.
16. Arifin WN, Zahiruddin WM. Sample size calculation in animal studies using resource equation approach. *Malays J Med Sci* 2017;24(5):101–105. doi:10.21315/mjms2017.24.5.11.
17. Badescu M, Badulescu O, Badescu L, Ciocoiu M. Effects of *Sambucus nigra* and *Aronia melanocarpa* extracts on immune system disorders within diabetes mellitus. *Pharm Biol* 2015;53(4):533–539. doi:10.3109/13880209.2014.931441.
18. Ciocoiu M, Badescu M, Badulescu O, Badescu L. The beneficial effects on blood pressure, dyslipidemia and oxidative stress of *Sambucus nigra* extract associated with renin inhibitors. *Pharm Biol* 2016;54(12):3063–3067. doi:10.1080/13880209.2016.1207088.
19. Seymenska D, Teneva D, Nikolova I, Benbassat N, Denev P. *In vivo* anti-inflammatory and antinociceptive activities of black elder (*Sambucus nigra* L.) fruit and flower extracts. *Pharmaceuticals* 2024;17(4):409. doi:10.3390/ph17040409.
20. Salvador ÂC, Rocha SM, Silvestre AJD. Lipophilic phytochemicals from elderberries (*Sambucus nigra* L.): influence of ripening, cultivar and season. *Ind Crops Prod* 2015;71:15–23. doi:10.1016/j.indcrop.2015.03.082.
21. Salvador ÂC, Król E, Lemos VC et al. Effect of elderberry (*Sambucus nigra* L.) extract supplementation in STZ-induced diabetic rats fed with a high-fat diet. *Int J Mol Sci* 2016;18(1):13. doi:10.3390/ijms18010013.
22. International Continence Society [Internet]. [cited 2025 Dec 1]. Available from: https://www.ics.org/glossary/diagnosis/interstitialcystitis.
23. Parsons CL. Advances in the treatment of interstitial cystitis. *Expert Opin Pharmacother* 2006;7(4):411–419. doi:10.1517/14656566.7.4.411.
24. Palumbo S, Lucarelli G, Lasorsa F et al. Urobiome and inflammation: a systematic review on microbial imbalances and diagnostic tools for urinary disorders. *Urology* 2025;200:206–215. doi:10.1016/j.urology.2025.03.050.
25. Ferreira SS, Martins-Gomes C, Nunes FM, Silva AM. Elderberry (*Sambucus nigra* L.) extracts promote anti-inflammatory and cellular antioxidant activity. *Food Chem X* 2022;15:100437. doi:10.1016/j.fochx.2022.100437.
26. Schwaiger S, Zeller I, Pölzelbauer P et al. Identification and pharmacological characterization of the anti-inflammatory principal of the leaves of dwarf elder (*Sambucus ebulus* L.). *J Ethnopharmacol* 2011;133(2):704–709. doi:10.1016/j.jep.2010.10.049.
27. Subbaramaiah K, Michaluart P, Sporn MB, Dannenberg AJ. Ursolic acid inhibits cyclooxygenase-2 transcription in human mammary epithelial cells. *Cancer Res* 2000;60(9):2399–2404. doi:10.1074/jbc.273.34.21875.
28. Ahmed SMU, Luo L, Namani A, Wang XJ, Tang X. Nrf2 signaling pathway: pivotal roles in inflammation. *Biochim Biophys Acta Mol Basis Dis* 2017;1863(2):585–597. doi:10.1016/j.bbadis.2016.11.005.
29. Lin P, Hwang E, Ngo HTT, Seo SA, Yi TH. *Sambucus nigra* L. ameliorates UVB-induced photoaging and inflammatory response in human skin keratinocytes. *Cytotechnology* 2019;71(5):1003–1017. doi:10.1007/s10616-019-00342-1.
30. Yamada T, Murayama T, Mita H, Akiyama K. Subtypes of bladder mast cells in interstitial cystitis. *Int J Urol* 2000;7(8):292–297. doi:10.1046/j.1442-2042.2000.00197.x.
31. Akiyama Y, Maeda D, Morikawa T. et al. Digital quantitative analysis of mast cell infiltration in interstitial cystitis. *Neurourol Urodyn* 2018;37(2):650–657. doi:10.1002/nau.23365.
32. Nordling J, Anjum FH, Bade JJ et al. Primary evaluation of patients suspected of having interstitial cystitis (IC). *Eur Urol* 2004;45(5):662–669. doi:10.1016/j.eururo.2003.11.021.
33. Lamale LM, Lutgendorf SK, Zimmerman MB, Kreder KJ. Interleukin-6, histamine, and methylhistamine as diagnostic markers for interstitial cystitis. *Urology* 2006;68(4):702–706. doi:10.1016/j.urology.2006.04.033.
34. Bhangoo S, Ren D, Miller RJ et al. Delayed functional expression of neuronal chemokine receptors following focal nerve demyelination in the rat: a mechanism for the development of chronic sensitization of peripheral nociceptors. *Mol Pain* 2007;3:38. doi:10.1186/1744-8069-3-38.
35. Tyagi P, Killinger K, Tyagi V, Nirmal J, Chancellor M, Peters KM. Urinary chemokines as noninvasive predictors of ulcerative interstitial cystitis. *J Urol* 2012;187(6):2243–2248. doi:10.1016/j.juro.2012.01.034.
36. Tseng-Rogenski S, Liebert M. Interleukin-8 is essential for normal urothelial cell survival. *Am J Physiol Renal Physiol* 2009;297(3):F816–F821. doi:10.1152/ajprenal.90733.2008.
37. Jiang YH, Jhang JF, Hsu YH, Ho HC, Wu YH, Kuo HC. Urine cytokines as biomarkers for diagnosing interstitial cystitis/bladder pain syndrome and mapping its clinical characteristics. *Am J Physiol Renal Physiol* 2020;318(6):F1391–F1399. doi:10.1152/ajprenal.00051.2020.
38. Erickson DR, Tomaszewski JE, Kunselman AR et al. Urine markers do not predict biopsy findings or presence of bladder ulcers in interstitial cystitis/painful bladder syndrome. *J Urol* 2008;179(5):1850–1856. doi:10.1016/j.juro.2008.01.047.
39. Korkmaz ME, Kart H, Muderrisoglu H. Tumor necrosis factor: a new mediator in the pathogenesis of myocardial ischemia syndromes. *Turk Kardiyol Dern Ars* 1995;23:380–383. (In Turkish).
40. Jin XW, Wang QZ, Zhao Y et al. An experimental model of the epithelial to mesenchymal transition and pro-fibrogenesis in urothelial cells related to bladder pain syndrome/interstitial cystitis. *Transl Androl Urol* 2021;10(11):4120–4131. doi:10.21037/tau-21-392.
41. Saban R. Angiogenic factors, bladder neuroplasticity and interstitial cystitis-new pathobiological insights. *Transl Androl Urol* 2015;4(5):555–562. doi:10.3978/j.issn.2223-4683.2015.08.05.
42. Abreu-Mendes P, Costa A, Charrua A, Almeida Pinto R, Cruz F. The role of urinary VEGF in observational studies of BPS/IC patients: a systematic review. *Diagnostics* 2022;12(5):1037. doi:10.3390/diagnostics12051037.
43. Chidlow JH Jr, Shukla D, Grisham MB, Kevil CG. Pathogenic angiogenesis in IBD and experimental colitis: new ideas and therapeutic avenues. *Am J Physiol Gastrointest Liver Physiol* 2007;293(1):G5–G18. doi:10.1152/ajpgi.00107.2007.
44. Trama F, Illiano E, Marchesi A et al. Use of intravesical injections of platelet-rich plasma for the treatment of bladder pain syndrome: a comprehensive literature review. *Antibiotics* 2021;10(10):1194. doi:10.3390/antibiotics10101194.