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ARAŞTIRMA MAKALESİ / RESEARCH ARTICLE

Morphometric Changes in Liver and Pancreas in Experimental Colitis Model and Examination of the Effects of Vagal Stimulation on These Changes in Chronic Period

Deneysel Kolit Modelinde Karaciğer ve Pankreasta Görülen Morfometrik Değişiklikler ve Vagal Stimulasyonun Kronik Dönemde Bu Değişiklikler Üzerine Olan Etkilerinin İncelenmesi

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ABSTRACT

Introduction: Inflammatory bowel disease is a chronic and idiopathic disease of the digestive tract. The disease also affects the liver and pancreas. Our aim in the study was to examine the effect of transcutaneous auricular vagal nerve stimulation (TAVNS) on the healing of liver and pancreas damage.

Material and Method: 36 rats in 4 groups were included in this study. The Sham group was intracolonic injected with saline and TAVNS was not applied. The Sham+ TAVNS group was injected intracolonically with saline and TAVNS was applied. The TNBS+Sham group was injected with TNBS (trinitrobenzene sulfonic acid) intracolonically and TAVNS was not applied. In TNBS+ TAVNS group, both TNBS was injected and TAVNS was applied. Liver tissue and pancreas tissue were examined histologically and histomorphometrically.

Results: In our study, the final body weights of TNBS+Sham and TNBS+ TAVNS groups were found to be significantly lower than Sham and Sham+TAVNS groups. The liver and pancreas histopathological scores of the TNBS injected groups were significantly higher. In the liver hepatocytes of rats in TNBS+Sham group, necrotic areas, vacuolar degeneration, and sinusoidal congestion were observed in some regions. Degenerative findings in liver sections of group TNBS+ TAVNS group were also partially reduced. The number and area of Langerhans islets in the pancreas of the animals in TNBS+Sham and TNBS+ TAVNS groups were found to be lower than in Sham and Sham+ TAVNS groups.

Conclusion: In this study, we found that TNBS-induced colitis in rats caused histopathological and histomorphometric changes in the liver and pancreas, causing weight loss, and that TAVNS had therapeutic effects on these changes.

Keywords: Colitis, vagal nerve stimulation, liver, pancreas

ÖZ

Giriş: İnflamatuvar bağırsak hastalığı, sindirim sisteminin kronik ve idiyopatik bir hastalığıdır. Hastalık ayrıca karaciğeri ve pankreası da etkilemektedir. Çalışmadaki amacımız transkutanoz aurikular vagal sinir stimulasyonunun (TAVNS) karaciğer ve pankreas hasarının iyileşmesi üzerindeki etkisini incelemektir.

Materyal ve Metod: Çalışmamız 4 grup halinde 36 sıçanla yapılmıştır. Sham grubuna intrakolonik salin enjeksiyonu yapılmış ve TAVNS uygulanmamıştır. Sham+ TAVNS grubuna intrakolonik olarak salin enjekte edilmiş ve TAVNS uygulanmıştır. TNBS+Sham grubuna intrakolonik olarak TNBS (trinitrobenzen sülfonik asit) enjekte edilmiş ve TAVNS uygulanmamıştır. TNBS+TAVNS grubuna hem TNBS enjekte edilmiş hem de TAVNS uygulanmıştır. Karaciğer dokusu ve pankreas dokusu histolojik ve histomorfometrik olarak incelenmiştir.

Bulgular: Çalışmamızda TNBS+Sham ve TNBS+TAVNS gruplarının son vücut ağırlıkları Sham ve Sham+TAVNS gruplarına göre anlamlı olarak düşük bulundu. TNBS uygulanan grupların karaciğer ve pankreas histopatolojik skorları anlamlı olarak yüksekti. TNBS+Sham grubundaki sıçanların karaciğer hepatositlerinde bazı bölgelerde nekrotik alanlar, vakuolar dejenerasyon ve sinüzoidal konjesyon gözlendi. Grup TNBS+TAVNS grubunun karaciğer kesitlerindeki dejeneratif bulgular kısmen azaldı. TNBS+Sham ve TNBS+TAVNS gruplarındaki hayvanların pankreasındaki Langerhans adacıklarının sayısı ve alanı, Sham ve Sham+ TAVNS gruplarına göre daha düşük bulundu.

Sonuç: Bu çalışmada sıçanlarda TNBS kaynaklı kolitin karaciğer ve pankreasta histopatolojik ve histomorfometrik değişikliklere neden olarak kilo kaybına yol açtığı ve TAVNS'nun bu değişiklikler üzerinde terapötik etkilerinin olduğu tespit edilmiştir.

Anahtar Sözcükler: Kolit, vagal sinir stimulasyonu, karaciğer, pankreas

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Introduction

Inflammatory bowel disease (IBD) is a chronic autoimmune condition whose exact cause remains unknown. It encompasses two primary types: ulcerative colitis and Crohn's disease. IBD is quite common in the world, and its incidence is increasing (1). While the exact cause remains incompletely understood, it is recognized that both genetic predisposition and environmental influences play significant roles in the development of the disease (2-4). Ulcerative colitis is characterized by inflammation restricted to the colon, whereas Crohn's disease can affect any segment of the gastrointestinal tract, often manifesting in the small and large intestines (5). Most of the clinical findings are similar in both types of IBD. Some of those are abdominal pain, diarrhea, and weight loss (6). Although this disease is predominant, especially in the intestines, extraintestinal findings are also seen in a significant part of the patients. Studies have reported that IBD also affects the liver and pancreas (7, 8). In treatment, medical and surgical methods keep the disease in remission for a while and suppress the symptoms, but side effects and long-term recurrence are common. For all these reasons, non-invasive, side-effect-free, easily applicable methods are needed in the treatment of IBD (9, 10). The experimental colitis model induced by TNBS (trinitrobenzene sulfonic acid), which we used in our study, is similar to human ulcerative colitis and is used in IBD research (11).

The vagus nerve holds the distinction of being the longest cranial nerve in the human body, coming out of the brain stem and extending to the thorax and abdomen, providing parasympathetic innervation to the organs here. It has special importance because it connects with both the central nervous system and the enteric nervous system. Recent research has stated that the vagus nerve provides an anti-inflammatory effect in cases of chronic inflammation through its wide neuronal network in the brain and afferent and efferent fibers extending to the body (12,13). The curative effect of the vagus nerve has been known since ancient times, and many methods have been used to stimulate the vagus nerve until today (14). Transcutaneous auricular vagal nerve stimulation (TAVNS) is a method based on stimulating the auricular branch, the only cutaneous branch of the vagus nerve, with electrical impulses over the skin. This method has received US Food and Drug Administration (FDA) approval for resistant epilepsy. Furthermore, the effectiveness of TAVNS method in treating many diseases such as depression, migraine, rheumatoid arthritis, and cardiac insufficiency is still being investigated. The fact that it is non-invasive, easily applicable, portable, and has minimal side effects makes this method advantageous (15).

In this research, our aim was to assess alterations in the liver and pancreas within a rat model of experimental colitis, as well as investigate the impacts of TAVNS on these changes during the chronic phase using histomorphometric and histopathological analysis.

Material and Methods

This research was carried out at Gazi University Experimental Research and Animal Laboratory. Ethical approval of the study was obtained from Animal Research Committee at Gazi University (G.Ü.ET 21.041). The study received support from the Gazi University Scientific Research Projects Unit under project number TTU-2021-7301. 36 adult male Sprague-Dawley rats, weighing between 180-200 g, were utilized for the study and maintained under controlled conditions. The temperature was maintained at 22±1°C, with humidity levels between 60-70%. The animals were subjected to a 12-hour light and 12-hour dark cycle. Adequate provisions were made to ensure that the rats had ad libitum access to food and water. They were housed in groups of three in cages. Prior to the commencement of the experiments, the rats were allowed to acclimate to these conditions for 7 days.

Experimental Groups

Four major groups of 9 rats each were created randomly from the total population of the rats. Animals in the Sham group received an intracolonic saline injection and TAVNS was not performed. Animals in Sham+TAVNS group received an intracolonic saline injection and TAVNS. Animals in Group TNBS+Sham group were injected with intracolonic TNBS and not TAVNS. Animals in TNBS+TAVNS group received intracolonic TNBS injection and TAVNS.

Colitis induction in TNBS+Sham and TNBS+TAVNS groups was applied according to the classical TNBS (trinitrobenzene sulfonic acid) colitis model developed by Morris et al. (11). The rats, after 12 hours of food deprivation, were anesthetized using a combination of 50 mg/kg ketamine and 0.05 mg/kg acepromazine. An 8 cm long cannula was inserted through the anus into the colon and TNBS was dripped in 50% ethanol (total volume, 0.25 ml) at 10 mg per rat (Figure 1). The rats were kept in an upside-down position so that TNBS could remain in the colon after intracolonic administration (Figure 2)

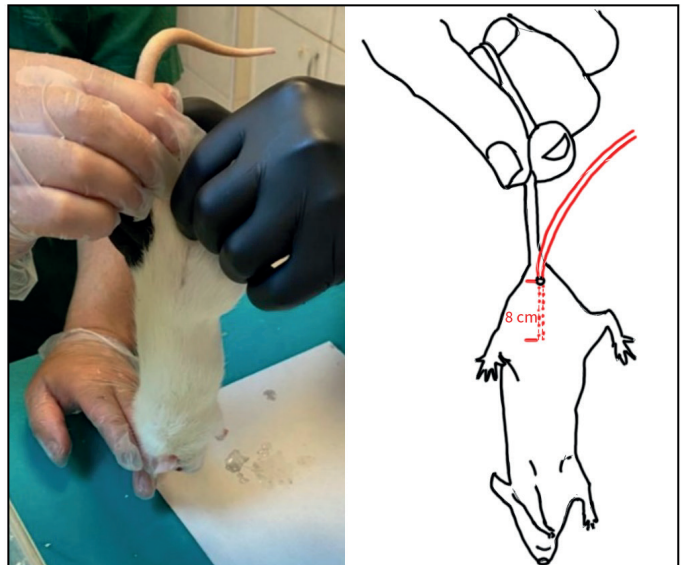

Figure 1. Colitis induction

Transcutaneous Auricular Vagal Nerve Stimulation (TAVNS)

The Vagustim® device, created for use in animal trials, was used to apply TAVNS. Vagustim was developed to non-invasively and bilaterally stimulate the auricular branch of the vagus nerve. It consists of two units, the stimulator, and the ear electrodes. While the stimulator generates electrical impulses, the ear electrode transfers the stimulus through the skin to the vagus nerve in both ears (16).

TAVNS was applied to the rats in Sham+TAVNS group and TNBS+TAVNS group under anesthesia for 30 minutes twice a day for 10 days. The ear electrodes of the device were placed in the tragus of the rats bilaterally, including the cavum concha, and stimulation was applied (Figure 3 and Figure 4). The same procedure was applied to Sham group and TNBS+Sham group but no stimulation. In our study, TAVNS current intensity was 1 mA, pulse duration was 500 µs, the frequency was 10 Hz, the voltage was 5 V, and the application was made accordingly. The application time continued for 30 minutes. Stimulation was conducted in cycles consisting of 30 seconds of stimulation followed by 30 seconds of rest. Consequently, each session involved a total of 15 minutes of vagal nerve stimulation.

Surgical Procedure

Rats were weighed before colitis induction and after 10 days of vagal nerve stimulation. On the 10th day after colitis induction, rats were deeply anesthetized using intramuscularly (ketamine 150 mg/kg and acepromazine 0.15 mg/kg). Then, rats were transcardially perfused with saline. Liver and pancreas tissues were removed. The liver and pancreas were weighed.

Histological Method

The liver and pancreas tissues of the experimental groups were fixed with 4% paraformaldehyde for light microscopic examination. Sections of 4 µm thickness were taken with a microtome (RM 2245, Germany) from paraffin blocks obtained after histological follow-up procedures. Tissues were stained with Hematoxylin-Eosin and Masson's Trichrome.

Liver and pancreas sections were stained in Harris Hematoxylin solution. Histopathological scoring was performed in the liver and pancreas tissues, and the area of the central vein in the liver and the area of Langerhans islets, and the number of Langerhans islets in the pancreas tissues were determined. According to the liver inflammation score, 0 points were given if there was no inflammation, score 1 if there was mild lobular/mild portal inflammation, score 2 if there was moderate lobular/portal inflammation, and score 3 if there was severe lobular/portal inflammation (17). According to the liver necrosis score, 0 points if there is no necrosis, score 1 if there is hepatic parenchymal necrosis below 10%, score 2 if 10-25% liver parenchymal necrosis is present, and score 3 if there is liver parenchymal

Figure 2. Upside-down position

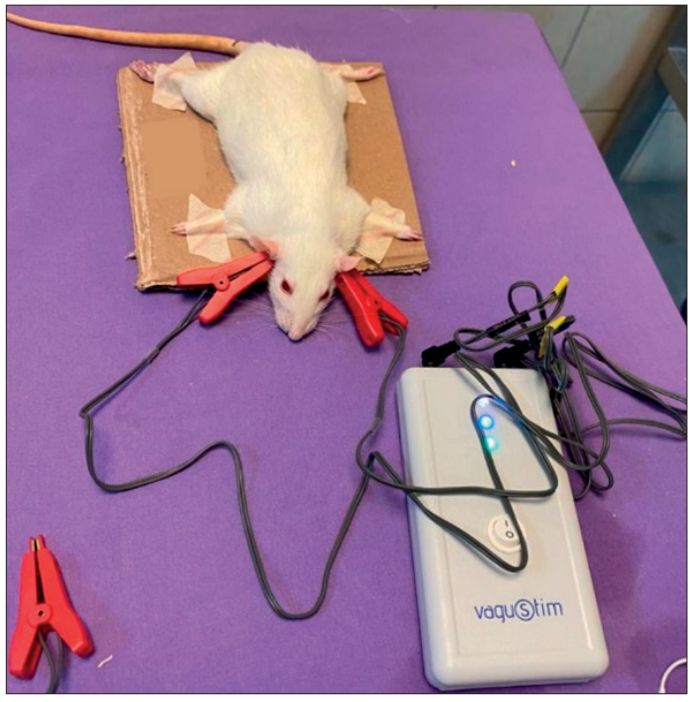

Figure 3. Transcutaneous auricular vagal nerve stimulation

Figure 4. Placement of the ear electrode

Table 1. Weight averages of the experimental groups

Mean±SD (standard deviation)	Sham Group Saline / TAVNS (-)	Sham+TAVNS Group Saline / TAVNS (+)	TNBS+Sham Group TNBS / TAVNS (-)	TNBS+TAVNS Group TNBS / TAVNS (+)
Final body weight (kg)	196.789±5.17	200.125±30.32	161.667±24.56	167.333±18.83
Liver weight (g)	7.397±0.61	9.446±1.20	6.583±0.87	9.030±1.36
Pancreas weight (g)	0.804±0.22	0.665±0.14	0.730±0.21	0.620±0.35

Table 2. Liver histological findings of the experimental groups.

Mean±SD	Sham Group Saline / TAVNS (-)	Sham+TAVNS Group Saline / TAVNS (+)	TNBS+Sham Group TNBS / TAVNS (-)	TNBS+TAVNS Group TNBS / TAVNS (+)
Liver Histopathological Score	0.4±0.50	0.7±0.57	2.45±0.82	2.05±0.60
Central vein diameter (μ)	55149±109	58325±132	53747±166	54134±135
Percentage of collagen fiber area (%)	2.79±2.6	3.19±2.3	10.28±6.9	7.27±5.7
Number of islets of Langerhans	13.71±5.02	12±4.69	5±1.86	6.83±3.18
Langerhans islet area (μ)	0.043±0.002	0.052±0.004	0.034±0.004	0.048±0.006
Pancreas histopathological score	0.5±0.51	0.5±0.51	2.3±1.03	2.05±0.60

necrosis above 25% (18). Pancreas histopathological scoring was conducted using parameters such as edema (rated on a scale of 0-4 points), acinar necrosis (0-4 points), hemorrhage and fat necrosis (0-4 points), as well as inflammation and perivascular infiltration (0-4 points), as outlined in a previous study (19). The mean scores of the rats were determined according to the groups.

Collagen fiber distribution was evaluated by performing Masson's Trichrome staining (Code: RRSK20-100, Atom Scientific, United Kingdom) on liver tissue sections of the experimental groups. The images captured using the Leica DM 4000B microscope from Germany were analyzed using the Leica LAS V4.9 program. The percentage of liver collagen fiber area was calculated utilizing Image J, a Java-based software program developed by the National Institutes of Health.

Statistical Analysis

SPSS (Statistical Package for Social Science, 22nd version) package program was used for statistical analysis of the obtained data. The normality of the data distribution was assessed using the Shapiro-Wilk test. A one-way analysis of variance (ANOVA) followed by the Tukey test was conducted. Results were deemed significant if the p-value was less than 0.05. In addition, the mean±standard deviation value was calculated.

Results

Weight Findings

At the end of the experiment, the final body weights of the rats were measured, and the average weight for each group was calculated. The mean weight of Sham group was found to be significantly higher than group TNBS+Sham group ($p<0.01$). The mean body weight of Sham+TAVNS group was higher than TNBS+TAVNS group ($p<0.05$). The mean liver weight of Sham group was found to be significantly lower than Sham+TAVNS group ($p<0.01$). The mean liver weight of TNBS+Sham group was significantly lower than TNBS+TAVNS group ($p<0.001$). Considering the mean pancreas weights of the rats; the mean pancreas weight of Sham group was higher than TNBS+Sham group, but the difference was not statistically significant ($p>0.05$), (Table 1).

Liver Histological Findings

In the liver sections examined after hematoxylin-eosin staining, cords consisting of polygonal hepatocytes extending radially from the central vein to the periphery and sinusoids between the cords were observed in the saline-injected groups (Sham and Sham+TAVNS groups). Hepatocytes with acidophilic staining had normal histological structure, some hepatocytes were observed to be binucleated. Liver sinusoidal spaces were in normal arrangement (Figure 5 A and B). In the group that received TNBS injection but did not receive TAVNS (TNBS+Sham group), inflammation in some lobular and portal areas was noted, as well as deterioration in hepatocyte structures and radial arrangement. Vacuolar degeneration and sinusoidal congestion were detected in certain areas of the hepatocytes. Localized necrotic areas were detected in the liver parenchyma (Figures 5 C and D). In the sections of both TNBS injection and TAVNS groups, as a result of histopathological scoring, it was observed that degenerative findings continued to exist even though they were partially reduced (Figure 5 E and F).

According to the histopathological scoring that determines the level of necrosis and inflammation in the livers of rats; the score of TNBS+TAVNS group was lower than TNBS+Sham group, but it was not statistically significant ($p>0.05$). The histopathological score of TNBS+Sham group was significantly higher than Sham group ($p<0.001$). The histopathological score of TNBS+TAVNS group was significantly higher than Sham+TAVNS group ($p<0.001$). There was no statistically significant difference between all groups in terms of liver central vein diameters of rats ($p>0.05$), (Table 2).

Following Masson's Trichrome staining, the percentage of collagen fiber area was determined. Subsequently, the collagen fiber area percentage was calculated for each group. Liver tissues were evaluated between groups, and no statistical significance was observed between the saline-injected groups (Sham and Sham+TAVNS groups). In the non-TAVNS treated groups, the collagen fiber distribution of those injected with TNBS was significantly lower than those injected with saline ($p<0.0001$). It was found that in the TNBS-injected groups, the percentage of collagen fiber area decreased in the non-TAVNS group compared to the TAVNS group, but this decrease was not statistically significant. In the TNBS-injected groups, the fibrotic changes observed in the livers of the non-TAVNS group (TNBS+Sham) were also partially reduced compared to the TNBS-injected and TAVNS-treated (TNBS+TAVNS) groups (Figure 6 A-F).

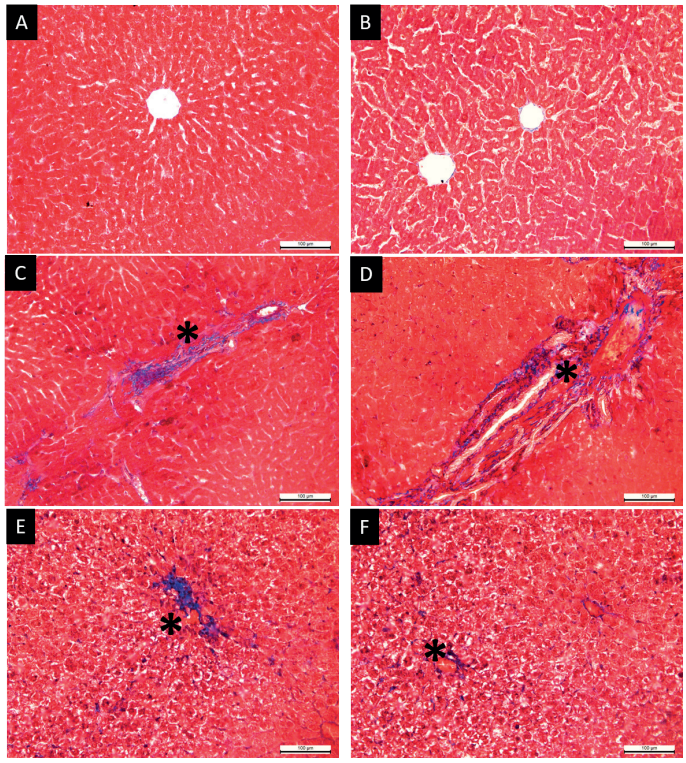

Figure 6. Liver tissue sections of the experimental groups were stained with Masson's Trichrome (MT). (A) Sham group; (B) Sham+TAVNS group, (C, D) TNBS+Sham group; (E, F) TNBS+TAVNS group, Collagen fibers (*) are seen as blue areas (MT, X200)

Pancreas Histological Findings

The exocrine section containing pancreas acini structures and endocrine areas including islets of Langerhans were observed in normal histological structure in pancreas sections stained with Hematoxylin-Eosin belonging to Sham group and Sham+TAVNS group. Interlobular connective tissue areas, intralobular canal structures, and vascularized formations had normal histological structures. While the islets of Langerhans were arranged as cords, the acini structures contained cells with basophilic cytoplasm at the base and eosinophilic cytoplasm at the apical (Figure 7 A and B).

In the exocrine pancreas tissue of TNBS+Sham group, acinar necrosis, hemorrhage, and perivascular infiltration were observed in some areas. In some islets of Langerhans, congestion between cells was noted. Lymphocytic infiltration was detected in interlobular areas, and atrophy was observed in some acinar cells in exocrine pancreas sections (Figures 7 C and D). It was noted that a decrease in acinar atrophy and lymphocytic infiltration was observed in sections belonging to TNBS+TAVNS group, and pancreas histopathological changes continued in some sections (Figure 7 E and F).

The average number of islets of Langerhans in the rat pancreas was calculated for each group. In the groups that did not undergo TAVNS, the saline-injected group exhibited significantly higher numbers of Langerhans islets compared to the TNBS-injected group ($p<0.01$). Within the TNBS-injected groups, although the TAVNS group showed a higher number of islets of Langerhans, the difference was not statistically significant ($p>0.05$). Regarding the average area of Langerhans islets in the rat pancreas, among the TAVNS groups, the TNBS-injected group displayed smaller islet areas compared to the non-TNBS-injected group.

Among the saline-injected groups, the islet areas in the group without TAVNS were observed to be lower than the group where TAVNS was applied. However, these differences

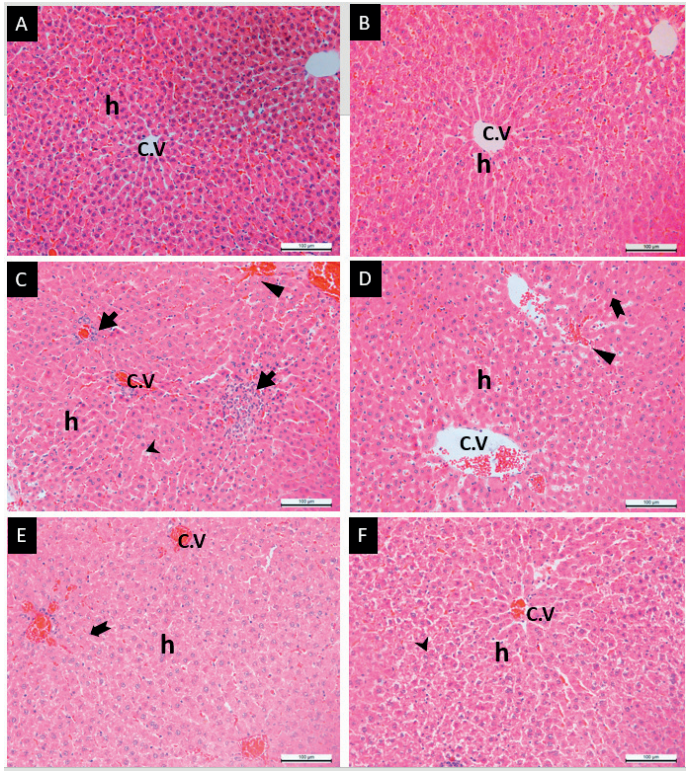

Figure 5. Liver tissue sections of the experimental groups stained with Hematoxylin-Eosin (HE); (A) Sham Group; (B) Sham+TAVNS Group, (C, D) TNBS+Sham Group; (E, F) TNBS+TAVNS Group, central vein (c.v), hepatocyte (h), inflammatory areas (➔), vacuolar degeneration (➤), necrosis (➡), congestion (▶) (HE, x200)

were not statistically significant (p>0.05). Regarding the histopathological scoring of pancreas tissues in rats, among the groups not subjected to TAVNS, the histopathological score of the TNBS injection group was markedly higher than that of the saline injection group (p<0.001). Similarly, among the TAVNS groups, the histopathological score of the TNBS injection group was significantly elevated compared to the saline injection group (p<0.001). (Table 2).

Discussion

Inflammatory bowel disease (IBD) is a prevalent, chronic disorder affecting the gastrointestinal tract, the exact cause of which remains uncertain. It encompasses two main types: ulcerative colitis and Crohn's disease, both of which can significantly impair quality of life and occasionally contribute to the development of depression and anxiety (20). There are many studies on the epidemiological, immunological, biochemical, and microbiological features of IBD (21). However, liver and pancreas involvement, which is one of the extraintestinal manifestations of the disease, has not been adequately studied although it is common.

Duan et al. (17) investigated the effects of a substance called vitexin on liver damage due to IBD in a study on mice. In the study, the colitis model induced by dextran sodium sulfate was used and the histopathology of the liver damage of the subjects was examined by Hematoxylin-Eosin staining. As a result of the study, it was stated that colitis causes inflammation and hepatocyte necrosis in the liver (22). In the study conducted by Lunder et al. (23), the researchers investigated the incidence of primary sclerosing cholangitis in patients with IBD. They examined the findings of primary sclerosing cholangitis 20 years later in 470 people diagnosed with IBD in Norway between 1990 and 1993. They stated that they detected primary sclerosing cholangitis in 7% of the patients, and they found biochemical markers indicating primary sclerosing cholangitis in 65% (23). In the research conducted by Değer et al. (24), the investigation centered around oxidative damage and apoptosis in rats with TNBS-induced colitis. The study also delved into the therapeutic potential of glutamine in mitigating damage to the colon and pancreas during colitis. A cohort of 28 male rats was randomly divided into four groups for the experiment. Group 1 received TNBS injection exclusively, Group 2 received TNBS injection along with glutamine supplementation, Group 3 received glutamine alone, and Group 4 received saline. It has been reported that interstitial edema and acinar vacuolization are observed in pancreas sections stained with Hematoxylin-Eosin and that glutamine treatment may have a protective effect on pancreas degeneration (24).

Today, IBD is tried to be treated with medical and surgical methods. However, both drug side effects and the recurrence of the disease create problems for patients and physicians.

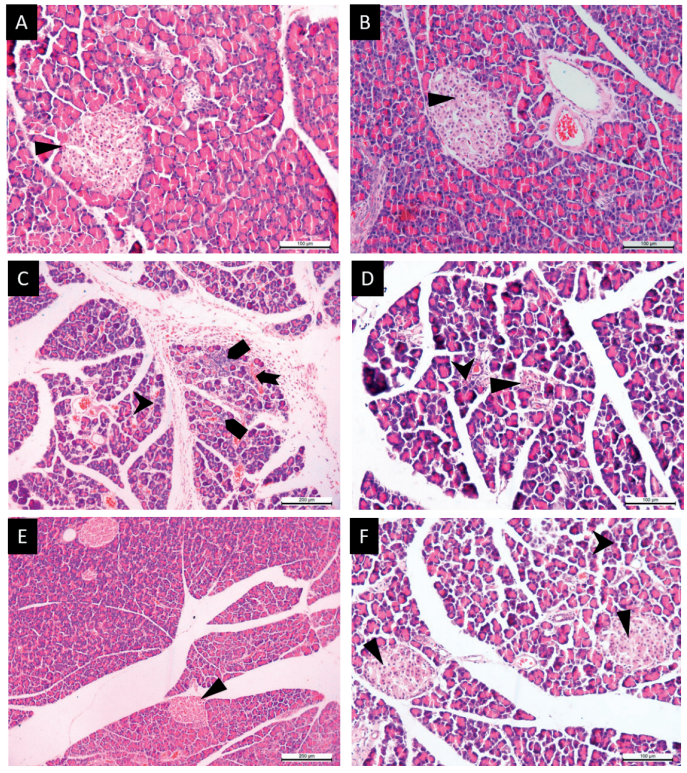

Figure 7. Images of the sections of the pancreas tissue stained with Hematoxylin-Eosin. (A) Sham group; (B) Sham+TAVNS group, (C, D) TNBS+Sham group; (E, F) TNBS+TAVNS group, Islets of Langerhans (►), atrophic acini (➤), perivascular infiltration (➡) and hemorrhage (➥) (HE, A, B, D, F: X200; C and E: X100)

Sympathetic system dominance is observed in 35% of people with Crohn's disease as a result of impaired parasympathetic activity (25). Recently, the focus has been on the vagus nerve's role in modulating inflammatory responses.

A study on mice by Ghia et al. (26) investigated the anti-inflammatory effect of the vagus nerve on colitis that persisted for several weeks. Using the dextran sodium sulfate-induced colitis model, vagotomy was performed in one group and not in the other group. When inflammatory markers and cytokine profiles were examined between groups, inflammation in vagotomized mice was more severe than in the control group. This indicates that the vagus nerve may have a protective role in IBD (26). Meregnani et al (27) investigated the effect of vagal nerve stimulation on IBD in TNBS-induced experimental colitis model in rats. Vagal nerve stimulation was performed with a stimulator surgically placed in the cervical region, and stimulation was performed 3 hours a day for 5 consecutive days. It has been reported that weight loss improved, and inflammatory markers were positively affected in rats that received vagal nerve stimulation (27). Rawat et al. (28), in a study on rats, investigated the effectiveness of TAVNS in 2-Dimethylhydrazine-induced colon cancer. In conclusion, it has been reported that vagal nerve stimulation of the transcutaneous auricular nerve improves autonomic function, reduces oxidative damage, and increases the activation of the

cholinergic anti-inflammatory pathway. TAVNS prevented the carcinogenic effects of 2-Dimethylhydrazine by increasing mitochondrial apoptosis (28).

Conclusion

In this study, it was observed that TNBS-induced colitis led to a reduction in body weight and an elevation in stress levels in rats. Examination of tissue sections stained with hematoxylin-eosin revealed inflammation in liver tissue, structural deterioration in hepatocytes, and necrosis in certain parenchymal areas due to colitis. While TAVNS was found to mitigate these degenerative changes, it did not completely prevent them. In the sections stained with Masson's Trichrome, fibrotic changes, and inflammatory areas were observed with the increase of collagen tissue in the liver tissue. Although not statistically significant, TAVNS reduced fibrotic changes in the liver. In the pancreas tissue, it has been observed that colitis reduces the number and area of Langerhans islets. This result suggested the deterioration in the endocrine functions of the pancreas. As a result of the histopathological scoring of the exocrine pancreas; exocrine pancreas degeneration was noted in the TNBS-injected groups. TAVNS reduced exocrine pancreas degeneration, but this effect was not statistically significant.

As a result, it is determined that TAVNS has a healing effect on the alterations that the IBD causes in the liver and pancreas. This research will support the treatment of IBD.

We predict that TAVNS is promising in IBD because it is easy to apply, non-invasive, has minimal side effects; and reveals an anti-inflammatory effect on the vagus nerve, but it should be supported by further experimental research.

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Yazar Katkıları: Fikir - MB, ÖK, RG, EA; Tasarım - ÖK, KA, SÖAD, EA; Denetleme - MB, RG; Veri Toplanması ve/veya İşlemesi -ÖK, EA, AS; Analiz ve Yorum - MB, RG, SÖAD; Literatür Taraması - ÖK, AS; Yazıyı Yazan - ÖK, KA, AS; Eleştirel İnceleme - MB, EA.

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