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Original Article

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Evaluation of Histopathological Effects of Acamprosate Use on Kidneys in Alcohol-Dependent Rats

Faik Özdengül[1], Burcu Gültekin[2], Hande Küsen[3], Behiye Nur Karakuş[3], Aysu Şen[1]

[1]Department of Physiology, Necmettin Erbakan University Meram Faculty of Medicine, Konya, Türkiye
[2]Department of Histology and Embryology, Necmettin Erbakan University Meram Faculty of Medicine, Konya, Türkiye
[3]Department of Physiology, Necmettin Erbakan University Institute of Health Sciences, Konya, Türkiye

ABSTRACT

Objective: Alcohol addiction is one of the growing global addiction threads. The present study aims to investigate histopathological effects of acamprosate, which is widely used in the treatment of alcohol dependence, on kidneys.
Methods: Rats were divided into 4 groups. The control group was given 10 mg/kg/day saline, and the alcohol group was given 10 mg/kg/day ethanol, diluted with 10 mg/kg/day saline. To the acamprosate group, 200 mg/kg/day acamprosate diluted with 10 mg/kg/day saline was given. The alcohol + acamprosate group was given 10 mg/kg/day ethanol diluted with 10 mg/kg/day saline, then combined with 200 mg/kg/day acamprosate. On the 21st day, after the study began, signs of alcohol withdrawal syndrome in the rats were evaluated. On the 22nd day, kidney tissues of the rats were extracted.
Results: Histopathological evaluation revealed that kidney tissues of the control group had normal structure. It was determined that Bowman's spaces were close to normal in kidneys of the alcohol group. In kidneys of the acamprosate group, an increased Bowman's space distance and intense tubular degeneration, shedding in tubule epithelial cells, and tubular dilatation were detected ($P < .05$). In kidneys of the alcohol + acamprosate group, Bowman's space distance was better than the acamprosate group, but tubular degeneration, shedding in tubule epithelial cells, and tubular dilatation continued ($P < .05$). Our findings revealed that the use of acamprosate alone produced serious histopathological consequences for kidneys.
Conclusion: It has been understood that it is important to control kidney health at certain intervals during the period of alcohol-dependent individuals without any kidney disease receiving acamprosate treatment.
Keywords: Acamprosate, acamprosate treatment, alcohol, kidney

Corresponding author: Hande Küsen ✉ handeksn@gmail.com
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INTRODUCTION

While the use of most addictive substances (heroin, cocaine, morphine, etc.) is prohibited by law, the use of alcohol is legal.[1] According to the World Health Organization, approximately 3 million people die every year due to alcoholism.[2] Alcoholism is also responsible for a sizable portion of the global burden of disease.[3,4]

In the treatment of alcoholism, both physiological and psychological dependence should be treated separately. While psychotherapy treatments are used for the treatment of psychological addiction, pharmacological agents are used for the treatment of physiological addiction.[4]

The number of people addicted to alcohol on a global scale is regularly increasing every year. For this reason, drugs used in the treatment of alcoholism are also becoming more and more common. Disulfiram, naltrexone, and acamprosate are among the drugs used in the treatment of alcoholism, but the most recent one of them is acamprosate.[5]

Acamprosate is a pharmacological agent approved by the Food and Drug Administration (FDA) in 2004. With the effectiveness of acamprosate, recurrence of alcohol consumption is reduced, and alcoholism is treated over time.[5-9] Other known and used names for acamprosate are acamprosate calcium and *N*-acetyl homotaurine .[8] After its use, acamprosate is absorbed by passive diffusion from small intestines and enters blood circulation.[10,11] Acamprosate functions as an NMDA (*N*-methyl-D-aspartate) receptor antagonist.[12,13] Acamprosate is removed from body via urine without being metabolized[6,14] and is one of the pharmacological agents that is widely used worldwide in the treatment of alcohol dependence.[15] Daily dose of acamprosate is determined by psychiatrists depending on current weight of person.[16] Acamprosate also has no interaction with alcohol. [14] Therefore, its use is considered safe.[10,17] Although the use of acamprosate is considered quite safe compared to other addictive agents, it also has various side effects.[9,18] Side effects seen are sexual aversion, nausea, abdominal pain, itching, vomiting, gas complaints, and skin rash.[17]

Although acamprosate is a pharmacological agent that is removed from body unchanged, it has been reported that it causes hydronephrosis and malformed iris formation in cases where dose adjustment cannot be maintained.[19,20]

In a study conducted in 2021, it is revealed that organ damage may occur as a result of combined use of naltrexone and acamprosate in alcohol dependent patients. Since naltrexone is metabolized by liver and acamprosate is excreted essentially unchanged by kidney, theoretically, no interacting organ toxicity is expected.[21] However, the use of acamprosate was stopped due to increase in the ureaand creatinine levels.[21]

In light of the findings obtained in experimental studies, the use of acamprosate in patients with kidney failure is considered to be contraindicated.[19,20,21] However, there is not enough information in the literature about the effects of acamprosate on kidneys after its use in healthy individuals. With the detection of changes in kidneys as a result of the use of acamprosate in healthy individuals, important information about current kidney functions of the users will be obtained, and an important step will be taken for the protection of kidney health in these patients.

Determining the pathological changes that occur as a result of the use of acamprosate is very important for success and sustainability of the applied treatment, which is the scope of our study.

MAIN POINTS

- Acamprosate is the most widely used pharmacological agent in the treatment of alcohol dependence.
- Acamprosate was found to cause an increase in the Bowman's space distance in the kidneys.
- Acamprosate has been found to cause extensive tubular degeneration and dilation of tubule cells.
- It was also determined that acamprosate formed a shedding in the tubule epithelium.
- It is important to keep kidney health under control during acamprosate use.

METHODS

Ethics committee approval for the study was obtained on November 12, 2021, with the number 2021-054. This study complies with World Medical Association's Declaration of Helsinki on ethical conduct of animal research.

In this study, a widely used 'Special Alcoholism Modeling for Acamprosate Use in Rats' model was utilized.[15,22] Thirty-two Wistar Albino female rats weighing 300-350 g were used. Rats were first randomly divided into 4 groups. According to the experimental model used, substance application procedures were carried out regularly for 21 days for all groups[22] (Table 1). Oral gavage approach was used in each procedure. During the experiment, fixed substances were administered to rats in the same group at the same time of each day. The control group (n = 8) received 10 mg/kg/day saline. The alcohol group (n = 8) received 10 mg/kg/day ethanol (99.8%) diluted with 10 mg/kg/day saline. The acamprosate group (n = 8) received 200 mg/kg/day acamprosate (Sigma-Aldrich/United States Pharmacopeia (USP) Reference Standard, 1000554-200MG) diluted with 10 mg/kg/day saline. In the alcohol + acamprosate group (n = 8), 10 mg/kg/day ethanol (99.8%) was first diluted with 10 mg/kg/day saline; then, 200 mg/kg/day acamprosate (Sigma-Aldrich) was added and applied.

Although the duration of alcohol dependence varies according to amount of alcohol used, it is widely accepted that it develops between 9 and 21 days in rats.[3] Emergence of alcohol withdrawal syndrome in rats reveals the existence of alcohol dependence.[3] Symptoms of alcohol withdrawal syndrome in rats are well described in the literature.[3] Withdrawal symptoms gradually worsen in parallel with time elapsed since last alcohol intake.[3] Various scoring systems are used to evaluate alcohol withdrawal syndrome in rats.[23] In our study, alcohol withdrawal syndrome scoring (EWS Score Test) was used. On the 21st day of the experiment, valid scoring method was applied after modeling processes were completed. Scoring was carried out in 4 groups. For scoring, rats were placed in transparent plexiglass cylinder observation cages with a diameter of 25 cm and a height of 65 cm. An independent researcher scored rats' alcohol withdrawal syndrome symptoms during a 10-minute follow-up session (10 minutes after 30 minutes after substance administration; 10 minutes after 120 minutes; 10 minutes after 240 minutes; and 10 minutes after 360 minutes, observing for a total of 4 scoring process was performed). A video recording system was used to record observation and scoring processes. As of 21st

Table 1. Substances Applied to the Experimental Groups for 21 Days and Their Amounts

	Group Names			
	Control Group	**Alcohol Group**	**Acamprosate Group**	**Alcohol + Acamprosate Group**
Substances applied	10 mg/kg/day saline	10 mg/kg/day ethanol + 10 mg/kg/day saline	200 mg/kg/day acamprosate + 10 mg/kg/day saline	10 mg/kg/day ethanol + 10 mg/kg/day saline + 200 mg/kg/day acamprosate

day of the experiment, item application phase of modeling was completed.

On the morning of 22nd day (08:00-08.20 AM), a mixture of ketamine hydrochloride (50 mg/kg) and xylazine (5 mg/kg) were administered intraperitoneally to fasted rats from night of the 21st day of the experiment. After anesthesia, cervical dislocation was performed and both right and left kidneys were removed. Kidneys were placed in formaldehyde and taken to laboratory for histopathological examinations. In histological examinations, kidney tissues taken from each group were placed in a 10% formaldehyde fixation solution and then kept for 24 hours and fixed. After the examination was made, kidney tissues were washed under tap water. Kidney tissues, which were washed under tap water for 24 hours, were then subjected to routine histological follow-up series. In the next step, kidney tissues were embedded in paraffin blocks. Then, sections of 5-6 µm thickness were taken from these paraffin blocks. Kidney tissues planned to be examined were stained with hematoxylin–eosin (H&E), Masson trichrome, and PAS (Periodic Acid Schiff) methods. In stained preparations, thickening of the basement membrane in glomerular structure; cell proliferation, and changes in epithelium was investigated. In addition, it was also examined whether there was enlargement due to degeneration and dilatation in kidney tubules. To determine the presence and severity of tissue damage, histopathological changes in the scanned microscopic areas were defined as congestion, hemorrhage, and edema. By severity of the damage observed, it was evaluated as: (−) if there is no tubule damage; (+, mild damage) if there is less than 10% ot the tubules damaged; (++, moderate damage) if 10% to 25% of the tubules damaged; (+++, extensive damage) if there was more than 25% of the tubules damaged. While determining damage, 8 slides were selected randomly from each group, and the relevant microscopic areas were evaluated by 2 independent histologists. The prepared slides were then examined and photographed under a research microscope (Necmettin Erbakan University Histology and Embryology Laboratory- Microscope Model Used in Imaging: Olympus BH-2)

Alcohol Withdrawal Syndrome Scores

Alcohol withdrawal syndrome scoring procedure was applied to 4 groups: control group, alcohol group, acamprosate group, and alcohol + acamprosate group.

Histological Examination Findings of Kidney Tissues

Hematoxylin–eosin, PAS, and Masson trichrome stains were used to determine appearance of rat kidneys in control and experimental groups as light microscopically. Kidney sections of groups were examined under a light microscope.

Statistical Analysis

Numerical data obtained were defined by the median, 25th and 75th percentiles and categorical data by frequency and percentage values. For statistical analysis of EWS Score Test data; Jamovi Version 2.3 (USA, PC software, retrieved from https://www.jamovi.org, 2022) and R Core Team Version 4.1 (GNU Public License, USA, PC software, retrieved from https://cran.r -project.org, 2021) programs were used. A negative binomial linear mixed effects model was performed to analyze group and time effects. Least squares means comparisons were done as post hoc comparisons. $P < .05$ was accepted as significant (Table 2).

Statistical comparisons between the groups were made by using the one- way analysis of variance(ANOVA) and Tukey's post hoc test after the data were checked for normality by using Kolmogorov–Smirnov (K–S) test (USA, PC software,2022). Comparisons for those that did not pass the normality test were made by using the Kruskal–Wallis test and Duncan's post hoc test. Comparisons and tests were performed using the GraphPad Prism 5.0 Demo software. Data are given as mean ± standard error, and $p < .05$ was chosen for statistical significance (Table 3).

Table 2. The Rat-Specific Alcohol Withdrawal Syndrome Behavioral Scoring Test (EWS Score Test)

Time (Minutes)	Control Group	Alcohol Group	Acamprosate Group	Alcohol + Acamprosate Group
30.	0 (0-0)	1 (1-1)*	0 (0-0)	1 (1-1)*
120	0 (0-0)	1 (1-1)*	0 (0-0)	1 (1-1)*
240	0 (0-0)	2 (2-2)*	0 (0-0)	1 (1-1)*
360	0 (0-0)	3 (3-3)*	0 (0-0)	2 (1-2)*

The data (median (25th percentile to 75th percentile)) obtained from the comparison of EWS scores with the control group at the specified times are included.
*$P < .01$.

Table 3. Statistical Evaluation of Histopathological Findings Between Groups

Groups	N	Mean ± STE (Standart Eror Value)
Control group	8	0.28 ± 0.18
Alcohol group	8	2.14 ± 0.26
Acamprosate group	8	1.28 ± 0.28
Alcohol + acamprosate group	8	1.42 ± 0.2

RESULTS

Alcohol Withdrawal Syndrome Results

As a result of the comparison of all rat groups with the control group in the relevant time periods; It was determined that the rats in alcohol group and alcohol + acamprosate group experienced alcohol withdrawal syndrome ($P < .01$). Alcohol and alcohol + acamprosate group rats were understood to be alcohol dependent (Table 2).

Hematoxylin–Eosin Staining Results

In the control group, it was observed that the appearance of tubule cells in the kidney cortex-medulla passage was normal, necrotic cells were not found in the kidney tubules, there was no blood supply between the tubule cells, and therefore, there was no pathological finding (Figure 1A). In the achol group, it was determined that Bowman's spaces were close to normal in the kidneys of alcohol-treated animals, but tubule cells showed marked vacuolization in places. It was observed that the cell nuclei of the tubular epithelium were located close to the lumen and some nuclei exhibited hydropic images (Figure 1B). In the acamprosate group, it was observed that the distance of the Bowman's space increased and intense tubular degeneration, shedding in the tubule epithelial cells and tubular dilatation were observed (Figure 1C). It was observed that the Bowman's space distance was better in the kidneys of the animals administered acamprosate with alcohol compared to the acamprosate group, but tubular degeneration, shedding of tubular epithelial cells and tubular dilatation continued (Figure 1D).

Periodic Acid Schiff Staining Results

In the histological examinations made on the sections prepared by the PAS method, it was observed that the brush edges with microvilli on the apical surfaces of the cells forming the proximal tubules in the cortex of the organ were PAS positive in both the experimental and control group rats (Figure 2A-D). It was observed that PAS positivity was less in the acamprosate group than in the other groups due to more intense tubular degeneration, especially the loss of microvilli structure.

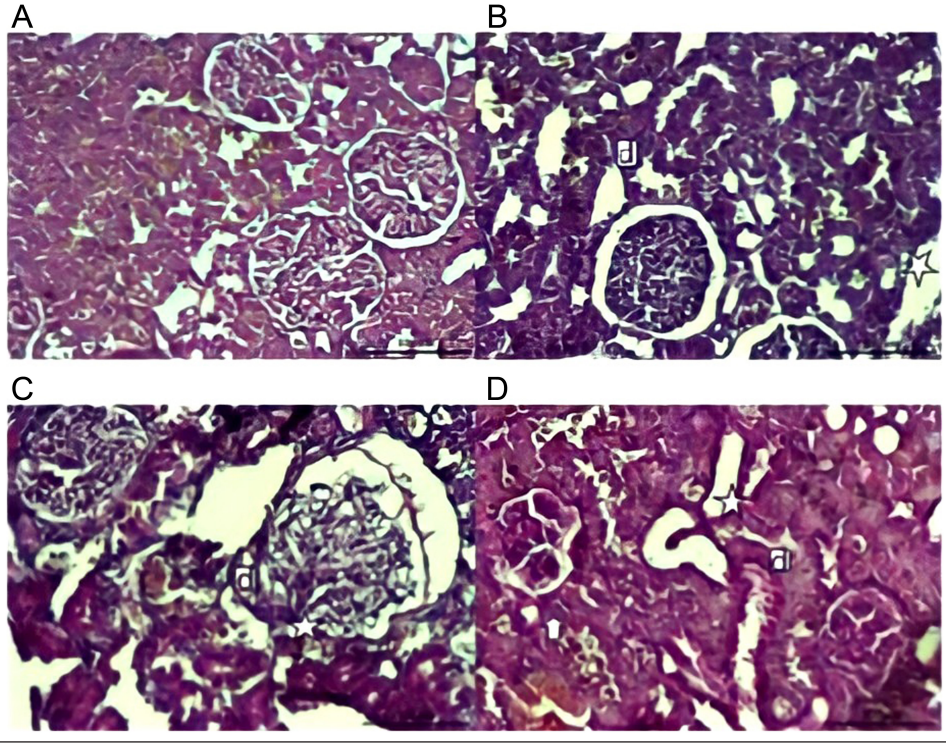

Figure 1. a-d. (A) Normal-looking glomerular structures, Bowman's capsule, range, and tubules in the kidney tissue section of the control group. (B) In the sections of the alchol group, glomeruli and Bowman's space (arrow), damage to tubular structures (degenerated epithelial cells (star) and dilatation (d)). (C) In the sections of the acamprosate group: significant glomerular damage (arrow), damage to tubular structures (degenerated epithelial cells (asterisk) and dilatation (d)). (D) In the kidney tissue section of the alcohol + acamprosate group: glomeruli and Bowman's space (arrow), damage to tubular structures (degenerated epithelial cells (asterisk) and dilatation (d)) (H&E). H&E, hematoxylin–eosin.

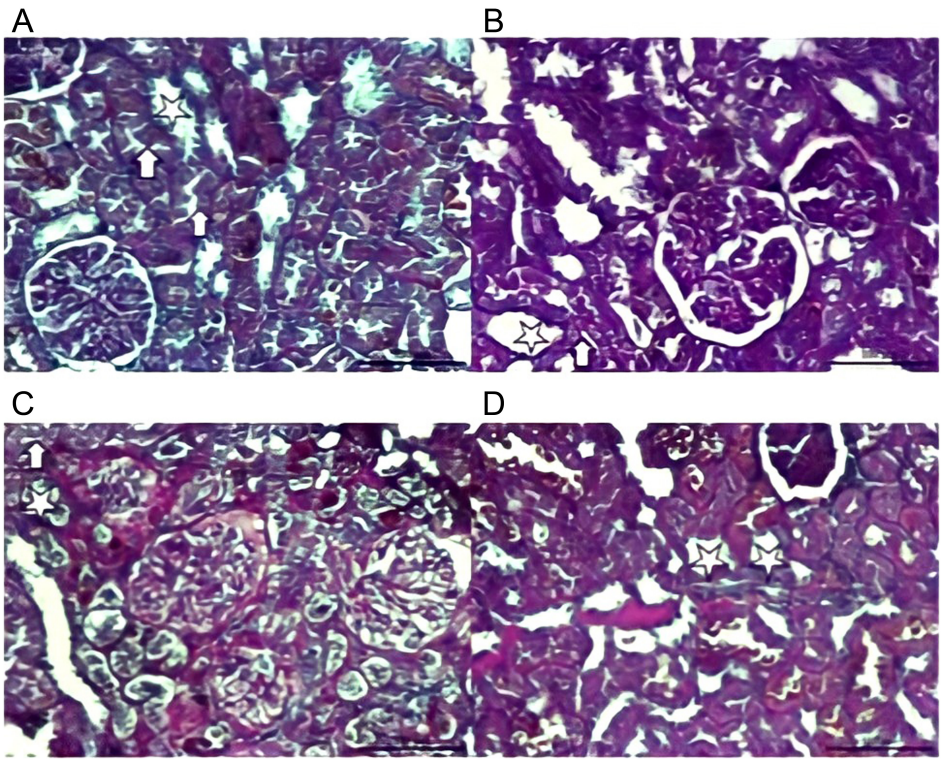

Figure 2. a-d. (A) Kidney tissue of the control group: arrows: brush borders with PAS-positive microvilli on the apical surfaces of the cells forming the proximal tubules, asterisks indicate the distal tubule. (B) In the sections of the alcohol group: arrow: brush edges with PAS-positive microvilli on the apical surfaces of the cells forming the proximal tubules, asterisks indicate the distal tubule. (C) In the kidney tissue sections of the acamprosate group: arrow: brush edges with PAS-positive microvilli on the apical surfaces of the cells forming the proximal tubules, asterisks show the distal tubule. (D) In the sections of the alcohol + acamprosate group: arrow: brush edges with PAS-positive microvilli on the apical surfaces of the cells forming the proximal tubules, asterisks indicate the distal tubule (PAS). PAS, periodic acid Schiff.

However, when the parietal leaf of the Bowman's capsule of the acamprosate group was compared to the other groups, PAS positivity was found to be higher (Figure 2C). In addition, PAS-positive granules were not observed in the cytoplasm of the cells forming the distal tubules in all groups (Figure 2A-D).

Masson Trichrome Staining Results

Collagen increase in glomeruli and tubulointerstitial area was evaluated with Masson trichrome stain. In the control group, amount of collagen was not increase in the glomerular and tubulointerstitial areas (Figure 3A). While minimal collagen accumulation increase was observed in the tubulointerstitial area in the alcohol group compared to the control group (Figure 3B), a significant collagen accumulation increase was observed in the tubulointerstitial area in the acamprosate group compared to the control group (Figure 3C). In the administered acamprosate together with the alcohol group, it was observed that there was no increase collagen accumulation in the tubulointerstitial area (Figure 3D).

Statistical Results of Histopathological Findings Between Groups

Statistically, there was a significant difference between the control group and alcohol group ($P < .05$), control group and acamprosate group ($P < .05$), control group and alcohol + acamprosate group ($P < .05$), alcohol group and alcohol + acamprosate group ($P < .05$), and alcohol group and acamprosate group ($P < .05$), but there was no difference between acamprosate group and alcohol + acamprosate group ($P > .05$) (Table 3).

DISCUSSION

As a result of alcohol addiction, physiological systems are negatively affected.[24] Disorders caused by alcohol addiction include balance disorders, nystagmus, corneal reflex loss, esophagitis, impaired bowel movements, alcoholic hepatitis, alcoholic cirrhosis, alcoholic cardiomyopathy, ischemic heart disease, and pancreatitis.[24] Approximately 3 million people die each year due to those disorders.[2]

On the global scale, the number of people addicted to alcohol is steadily increasing every year. This increase makes the treatment of alcohol addiction more important day by day.[25] The most recent agent used in the treatment of alcohol dependence is acamprosate.[17] Acamprosate functions as an NMDA (*N*-methyl-D-aspartate) receptor antagonist.[12,13] Acamprosate is removed from the body via urine by filtration through the kidneys while not being metabolized.[18] Acamprosate has no

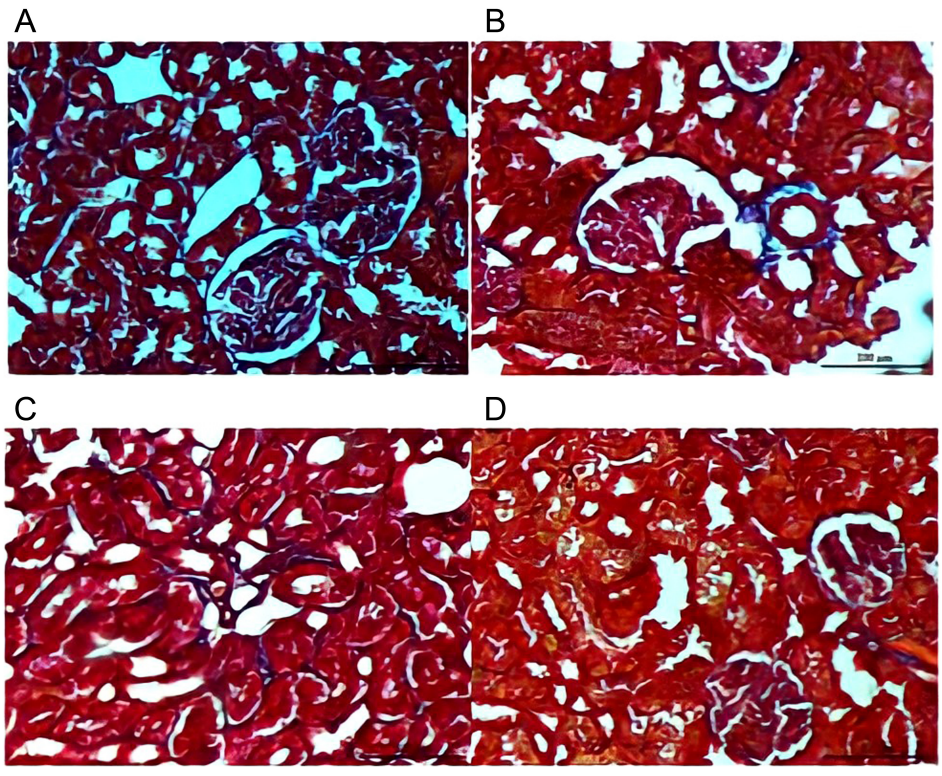

Figure 3. a-d. (A) Kidney tissue of the control group: normal glomeruli (arrow) and tubulointerstitial structure. (B) Alcohol group: slight collagen increase in the tubulointerstitial area (blue colored areas). (C) In the kidney tissue sections of the acamprosate group: collagen increase in the tubulointerstitial area (blue colored areas). (D) In the sections of the alcohol + acamprosate group: slight collagen increase in tubulointerstitial area (blue-colored areas) was evaluated with Masson trichrome stain.

interaction with alcohol.[12] For these reasons, use of acamprosate appears to be quite safe compared to other addiction agents. Although it is considered safe, use of acamprosate has various side effects.[9,18] These effects are sexual aversion, nausea, abdominal pain, itching, vomiting, gas complaints and skin rash.[17]

In a study conducted in 2021, alcohol addicts treated with acamprosate were not associated with serum enzyme elevations above the rates that occurred with placebo treatment.[26] Acamprosate is a synthetic amino acid, minimally metabolized in the liver and excreted largely unchanged in urine, probably due to a lack of hepatotoxicity.[27]

It has been reported that hydronephrosis and malformed iris formation occur in the case of failure to adjust the dose in the treatment of acamprosate.[19,20]

Before starting acamprosate treatment, general health status of the patient is subjected to various basic examinations.[16] The use of acamprosate is considered contraindicated in individuals with kidney impairment in pretreatment examinations.[16,28,29] There is no mandatory practice to evaluate kidney functions during the treatment process in individuals whose general health status is considered suitable for the use of acamprosate.

In the studies carried out, it was determined that the urea and creatinine values in the urine samples taken during the use of acamprosate in healthy individuals were found to be much higher than the normal.[21,28] In the light of the available data, the hypothesis that acamprosate may cause damage to the kidney tissues has occurred. The data obtained in our study reveal that damage to kidney tissues develops as a result of the use of acamprosate. It was also understood that the findings obtained in the study were in parallel with the other findings in the literature.

In the light of the findings obtained in our study, it has been understood that individuals whose general health status is considered suitable for acamprosate use should be evaluated by nephrology specialists at certain time periods during the treatment process and treatment should be continued accordingly.

CONCLUSION

According to our findings, it was determined that the use of acamprosate affected the kidney tissue pathologically. It was also understood that existing pathological effect was more severe in the group in which only acamprosate was used (when compared to the alcohol + acamprosate group). It was concluded that kidney functions should be carefully evaluated before using acamprosate, and kidney functions should be

re-evaluated at regular intervals during its use. New studies are needed to reveal pathological side effects of acamprosate use on kidney functions in more detail.

Ethics Committee Approval: Ethical committee approval was received from the Animal Experiments Local Ethics Committee of University of Necmettin Erbakan KONÜDAM Experimental Medicine Application and Research Center Directorate (Approval no: 2021-054, Date: November 12, 2021).

Informed Consent: N/A.

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