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# Acute Liver and Kidney Damage Due to Ethanol: The Impact of Disulfiram Treatment

## Etil Alkol Nedeniyle Oluşan Akut Karaciğer ve Böbrek Hasarı: Disülfiram Tedavisinin Etkisi

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## Abstract

**Objective:** Acute ethanol (EtOH) exposure is known to induce oxidative stress, inflammation, and cellular injury in various organs, particularly the liver and kidneys. Disulfiram (DSF), a drug used in alcohol cessation therapy, also exhibits anti-inflammatory and antioxidant properties. This study aimed to evaluate the extent of liver and kidney damage induced by acute EtOH exposure and to investigate the potential therapeutic effects of DSF using histopathological and immunohistochemical methods.

**Method:** Twenty-eight male Sprague-Dawley rats were randomly divided into four groups: Control, EtOH, EtOH+DSF, and DSF. The EtOH group received 5 g/kg EtOH orally, while the EtOH+DSF group additionally received 100 mg/kg DSF administered intraperitoneally. Tissue sections were stained with hematoxylin and eosin, Masson's trichrome, and periodic scid-schiff for semiquantitative evaluation. Interleukin-1beta (IL-1β) expression was assessed using avidin-biotin immunohistochemistry and quantified with ImageJ software.

**Results:** Histopathological examination revealed significant hepatocyte degeneration, inflammatory cell infiltration, and fibrosis in the EtOH group, along with tubular degeneration, hemorrhage, and structural disorganization in the kidneys. DSF treatment markedly ameliorated these histological alterations. Immunohistochemical analysis demonstrated that IL-1β expression was significantly increased following EtOH exposure but was notably reduced in the DSF-treated group.

## Öz

**Amaç:** Akut etanol (EtOH) maruziyetinin, özellikle karaciğer ve böbreklerde olmak üzere çeşitli organlarda oksidatif stres, enflamasyon ve hücresel hasara yol açtığı bilinmektedir. Alkol bırakma tedavisinde kullanılan bir ilaç olan disülfiram (DSF), aynı zamanda anti-enflamatuvar ve antioksidan özellikler de göstermektedir. Bu çalışma, akut EtOH maruziyetinin neden olduğu karaciğer ve böbrek hasarının boyutunu değerlendirmeyi ve DSF'nin olası terapötik etkilerini histopatolojik ve immünohistokimyasal yöntemler kullanarak araştırmayı amaçlamıştır.

**Yöntem:** Yirmi sekiz erkek Sprague-Dawley sıçanı rastgele dört gruba ayrılmıştır: Kontrol, EtOH, EtOH+DSF ve DSF. EtOH grubuna oral yolla 5 g/kg EtOH verilmiş, EtOH+DSF grubuna ise ek olarak 100 mg/kg DSF intraperitoneal yolla uygulanmıştır. Doku kesitleri yarı kantitatif değerlendirme için hematoksilen-eozin, Masson's trikom ve periyodik asit-schiff ile boyanmıştır. İnterlökin-1beta (IL-1β) ekspresyonu avidin-biotin immünohistokimya yöntemiyle değerlendirilmiş ve ImageJ yazılımı kullanılarak kantitatif olarak analiz edilmiştir.

**Bulgular:** Histopatolojik inceleme, EtOH grubunda belirgin hepatosit dejenerasyonu, enflamatuvar infiltrasyon ve fibrozis; böbreklerde ise tübüler dejenerasyon, hemoraji ve yapısal bozulma olduğunu ortaya koymuştur. DSF tedavisi bu histolojik değişiklikleri belirgin şekilde hafifletmiştir. İmmünohistokimyasal olarak, EtOH maruziyeti sonrasında IL-1β ekspresyonu anlamlı düzeyde artmış, ancak DSF tedavisi uygulanan grupta belirgin biçimde azalmıştır.

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## Abstract

**Conclusion:** These findings suggest that DSF exerts therapeutic and anti-inflammatory effects against EtOH-induced hepatic and renal injury, primarily through modulation of IL-1β-mediated inflammatory pathways.

**Keywords:** Disulfiram, ethanol, kidney, liver

## Öz

**Sonuç:** Bu bulgular, DSF'nin EtOH kaynaklı karaciğer ve böbrek hasarına karşı terapötik ve anti enflamatuvar etkilere sahip olduğunu ve bu etkinin büyük ölçüde IL-1β aracılı enflamatuvar yolların düzenlenmesiyle ilişkili olduğunu göstermektedir.

**Anahtar kelimeler:** Böbrek, disülfiram, etanol, karaciğer

## Introduction

Ethyl alcohol is one of the most widely used psychoactive substances worldwide. Chemically known as ethanol (EtOH), it is a colorless, flammable liquid with a slightly pleasant odor (1). It causes significant health problems through both chronic and acute exposure, contributing to morbidity and mortality at individual and societal levels. Acute EtOH exposure usually occurs as a result of consuming large amounts of alcohol in a short period of time (2). This exposure can affect many systems such as the central nervous system, gastrointestinal system, and cardiovascular system. It can also suppress the immune system and cause harm to a wide range of individuals. However, one of the most serious and common form of organ damage caused by EtOH occurs in the liver (1). Short-term high doses of EtOH disrupt hepatic metabolism and cause lipid accumulation (steatosis) in liver cells (3). Furthermore, acetaldehyde and reactive oxygen species (ROS) formed during the metabolism of alcohol cause oxidative stress in hepatocytes, leading to cell damage. This process can lead to apoptosis and necrosis of hepatocytes. The presence of necrotic cells triggers an inflammatory response (4). Acute EtOH exposure has also been found to have nephrotoxic effects on the kidneys. Histopathologic changes such as dilatation of glomerular capillaries, interstitial edema and tubular degeneration may lead to decreased glomerular filtration rate and electrolyte imbalances (5).

Interleukin-1beta (IL-1β) is a key proinflammatory cytokine that contributes significantly to inflammation. Whenever pathogens or tissue injury are present, IL-1β activates a signaling cascade that leads to a multi-phase inflammatory response at the cell level (6,7). Excessive EtOH exposure induces oxidative stress and cell death, leading to increased IL-1β levels. This elevation further exacerbates inflammation-associated tissue damage, particularly in organs such as the liver (8). Experimental data has shown that consumption of alcohol triggers the tumor necrosis factor-alpha (TNF-α)/nuclear factor Kappa B (NF-κB) signaling pathway in kidney tissue, which leads to alterations in the kidney that are linked to inflammation (9).

Disulfiram (DSF) is a drug used in the treatment of EtOH dependence that exerts its main effect by inhibiting the enzyme aldehyde dehydrogenase. It increases the level of acetaldehyde in the blood by preventing the metabolism of acetaldehyde produced after EtOH intake. This build-up leads to unpleasant symptoms such as dizziness, palpitations, nausea and flushing of the face, with the aim of getting the patient to stop drinking alcohol (10). In addition to its well-known aversive effects, DSF has recently been shown to have antioxidant and anti-inflammatory properties (11). Through lowering ROS, which are significant triggers of intracellular oxidative stress, DSF exhibits antioxidant properties. In this regard, DSF reduces oxidative cellular processes including DNA damage and lipid peroxidation (12). Furthermore, by blocking NF-κB and inflammasome signaling pathways, DSF lowers the synthesis of proinflammatory cytokines including IL-1β and TNF-α. By reducing the intensity of the inflammatory response, this impact might stop tissue damage from getting deeper (13). The goal of our investigation was to use histopathologic and immunohistochemical analysis to examine the impact of DSF, a compound with therapeutic potential, on the structural changes brought on by acute EtOH exposure in tissues.

## Materials and Methods

### Ethical Approval

All animal procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council). This study was reviewed by the Local Ethics Committee of Kırşehir Ahi Evran University Animal Experiments and this committee approved our experimental guidelines (decision no: 24/057, date: 19.01.2024).

### Animals and Experimental Groups

The Experimental Research Center of Kırşehir Ahi Evran University served as the site for this investigation. We used stress-free circumstances (21 °C; 12-h light/12-h dark cycles) to examine adult male Sprague-Dawley rats (n=28), which

were 8-10 weeks old and weighed 200-250 g. Four distinct experimental groups were created for this investigation, each with seven rats: Control, EtOH, EtOH+DSF, and DSF. A total of fifteen days were allocated for the investigation. At the conclusion of the fifteenth day, the control group was sacrificed by cervical dislocation and tissue and blood samples were taken under anesthesia without any type of treatment. EtOH group rats were given EtOH orally at a dose of 5 g/kg (14) three times a week, at 12-hour intervals/12-h intervals. Following EtOH administration, a 100 mg/kg dose of DSF (15) was diluted in 0.9% saline and given intraperitoneally once daily for seven days in the EtOH+DSF. The DSF group received only DSF at a dosage of 100 mg/kg (Table 1). All animals were euthanized by cervical dislocation at the end of the experimental period under general anesthesia with xylazine (10 mg/kg) and ketamine (60 mg/kg). Afterwards, the liver and kidney tissue were taken for histopathological and immunohistochemical analyses.

### Histopathological Evaluation

The tissue samples collected at the end of the experiment were kept in a 10% formaldehyde solution to histologically assess liver and kidney tissue changes in each experimental group. 72 hours of formaldehyde fixation, the tissues were rinsed under running water. Following a series of alcohol dehydrations, they were cleaned in xylene, embedded in paraffin, and paraffin blocks were formed. Rat liver and kidney tissue paraffin blocks were cut into sections 5 µm in thickness, which were then put on poly-L-lysine-coated slides. Sections were stained with hematoxylin & eosin, Masson trichrome (MT) and periodic acid-schiff (PAS). Following staining, the sections were handled by a series of increasing alcohol passes, xylene cleaning, entellan mounting on a coverslip, and light microscopy analysis (Nikon Eclipse Si, Tokyo, Japan). For histological scoring, ten randomly selected microscopic fields were evaluated in each section. Furthermore, liver tissue damage included hepatocyte degeneration and fibrosis, while renal tissue damage was assessed and scored for tubule degeneration and hemorrhage. These parameters were scored semiquantitatively for each criterion on a scale of 0 to 3 (0: None, 1: Mild, 2: Moderate, 3: Severe).

### Immunohistochemical Evaluation

The expression of IL-1β in liver and kidney tissue was shown by immunohistochemistry. For immunohistochemical staining, the avidin-biotin peroxidase assay was used. To stain, 5 µm slices of paraffin blocks were created on polylysine slides. Sections were deparaffinized with xylene, then preserved with a declining alcohol before their rehydration in distilled water. Sections were heated in a 600 W microwave oven with 5% citrate buffer for antigen retrieval, then rinsed with phosphate buffered saline (PBS) and treated with 3% $H_2O_2$ to suppress endogenous peroxidase activity. The immunohistochemical staining kit (Thermo Scientific/TS-125-HR, Thermo Fisher Scientific Inc., Waltham, MA, USA) was used in all subsequent steps, and the entire treatment carried out in a room that prevents tissue drying. Block serum was applied into PBS-washed sections for 10 minutes at room temperature to cover the areas external of the antigenic regions. The primary antibody for IL-1β (Proteintech, 26048-1-ap, 1:200) was then incubated at 4 °C overnight. Biotinylated secondary antibodies were subsequently used to incubate the sections. Streptavidin-peroxidase mixture was applied after washing with PBS. Following that was amino-9-ethylcarbazole (HA53704, Thermo Scientific, USA) that highlighted

**Table 1. Overview of the treatment protocols applied to each experimental group during the 15-day study period, including administered substances, dosages, routes, frequency, and duration**

| Group | Number of animals | Treatment | Dose | Route | Frequency | Duration |
|---|---|---|---|---|---|---|
| Control | 7 | No treatment | - | - | - | No treatment for 15 days; sacrificed on day 15 |
| EtOH | 7 | Ethanol | 5 g/kg | Oral gavage | Three times per week; two doses at 12-hour intervals on each administration day | During the 15-day experimental period |
| EtOH+DSF | 7 | Ethanol+disulfiram | EtOH: 5 g/kg DSF: 100 mg/kg | EtOH: oral DSF: intraperitoneal | EtOH: three times per week DSF: once daily | Ethanol administered first; DSF administered once daily for 7 consecutive days after ethanol exposure |
| DSF | 7 | Disulfiram | 100 mg/kg | Intraperitoneal | Once daily | 7 consecutive days during the experimental period |

DSF: Disulfiram, EtOH: Ethanol

the immunoreactivity. To boost nuclear staining, Gill's hematoxylin was used as a counterstain. Sections of liver and kidney stained with immunohistochemistry were examined under light microscopy, and microscopic images from randomly selected locations were acquired. Twenty distinct microscopic fields were assessed from each section for quantitative analysis. Immunoreactivity examination was conducted specifically in the pericentral (central vein-adjacent) regions of the liver tissue and in the cortical region of the kidney. ImageJ software was used for assessing the immunoreactivity intensities of the markers identified in the images. The overall immunoreactivity was evaluated using ImageJ software's color threshold plugin.

### Statistical Analysis

For all research involving statistics, Graphpad Prism version 9 was applied. The data distribution was determined using the Shapiro-Wilk test. The Kruskal-Wallis test and One-Way Analysis of Variance (ANOVA) were the methods used for comparisons involving more than two groups. Significant post-hoc comparisons of the variables were found using the Dunn test for the Kruskal-Wallis analysis and the Bonferroni test for the One-Way ANOVA. In all data, a p-value of less than 0.05 was accepted as statistically significant.

## Results

### Body Weight

Both before and after the experiment, the rats' body weights were measured. Figure 1 compares body weight changes among the groups. Rats in the EtOH group experienced a significant ($p<0.01$) weight loss at the conclusion of the experiment. Upon completion of the assessment, the weights of the DSF group ($p<0.01$) and the cntrol group ($p<0.01$) were both greater than their initial weights.

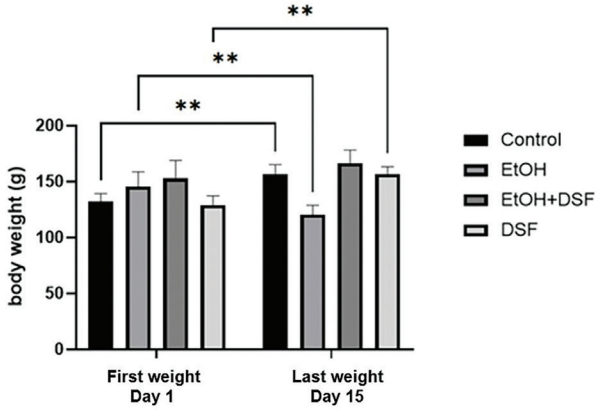


**Figure 1.** Comparison of body weights of rats belonging to all groups before and after the study

**: p<0.05, **: p<0.01, ***: p<0.001, DSF: Disulfiram, EtOH: Ethanol*

### Histopathology of Liver and Kidney Tissues

#### Liver Assessment

Figure 2A illustrates the histologic evaluation of liver tissues from all groups. Hepatocytes in the control group's histologic sections had a regular organization, homogenous cellular architecture, and a normal sinusoidal structure. The DSF group had a histologic appearance similar to the control group. There was healthy cellular integrity. When analyzing the control liver stained with MT, we observed minimal collagen deposition around the vessels (Figure 3A). PAS staining demonstrated a normal glycogen distribution in the hepatocyte cytoplasm (Figure 4A). The DSF group showed results that were similar to these assessments. In the EtOH group, hepatocytes degenerated ($p<0.001$) and vacuolized. This group's liver sections showed inflammatory cell infiltration, among additional findings. Necrotic cells were also found in a few locations (Figure 2A). Fibrosis development was observed in the MT-treated EtOH group due to alcohol toxicity ($p<0.001$) (Figure 3A). PAS staining revealed a reduction in PAS-positive staining intensity compared to the control group (Figure 4A). In the EtOH group treated with DSF, hepatocyte structure was found to be more regular, and inflammatory cells were diminished. There were also reduced regions of degeneration and fibrosis ($p<0.05$) (Figures 2-5, Table 2).

#### Kidney Assessment

When the kidney tissue was investigated, it show that the control group had a regular, round glomerulus structure with established boundaries. Tubule epithelial cells were normal, the lumen was visible, and the nuclei had a typical appearance. There were no significant pathologic findings in the DSF group, and the histologic structure was maintained. The basement membrane and tubule structure were comparable to the control group (Figure 2B). The connective tissue in the control group was found to be within normal boundaries by MT staining (Figure 3B). Furthermore, glomerular basement membranes were clearly visible and tubule brush borders were preserved in the control group, according to PAS staining (Figure 4B). The findings of the DSF group were similar to these assessments. In the EtOH group, the glomerular structure was retracted, and degenerative glomeruli with hypocellularity were observed. Tubular degradation was

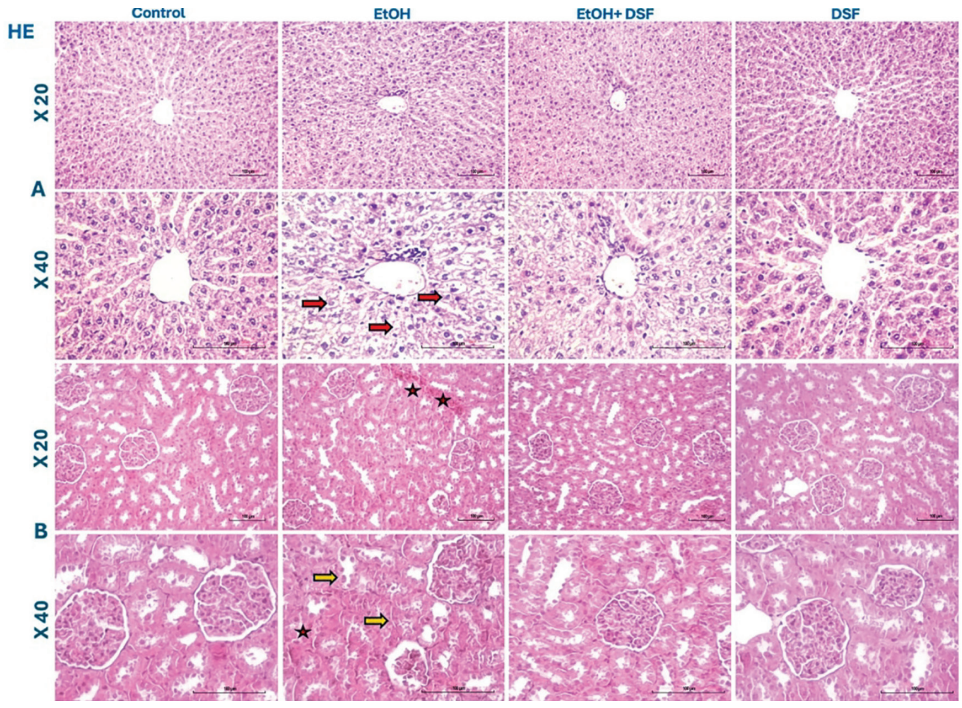


**Figure 2.** Light microscopic images of rat liver and kidney tissues stained with hematoxylin and eosin (H&E), (Nikon Eclipse Si, Tokyo, Japan. X200 and X400). Hepatocyte degeneration (red arrow), tubular degeneration (orange arrow) and hemorrhage (red star) in the experimental groups

*DSF: Disulfiram, EtOH: Ethanol*

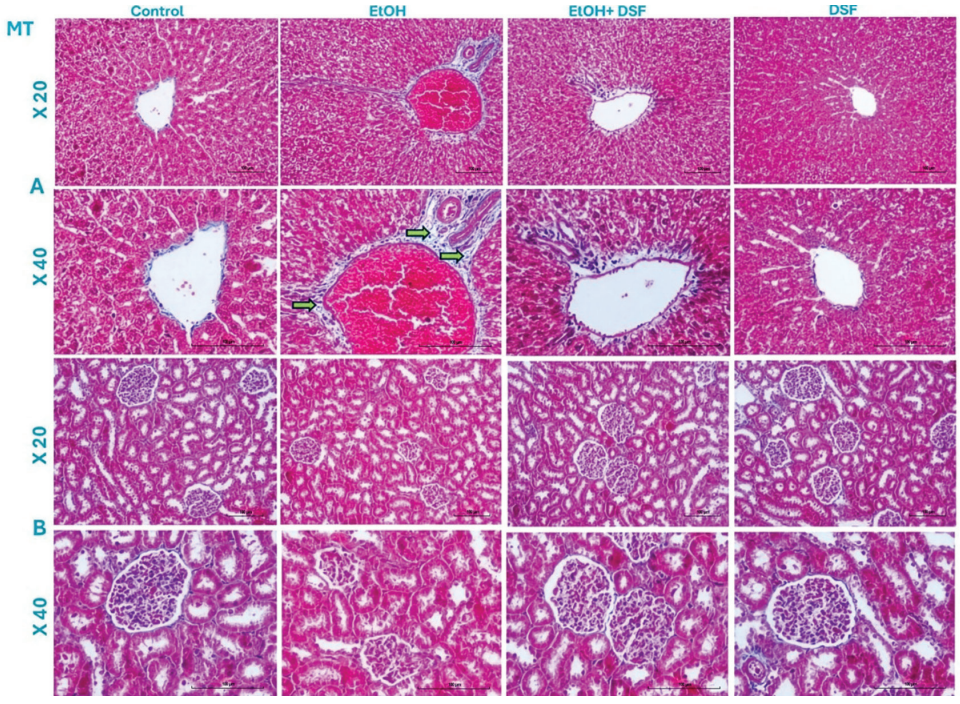


**Figure 3.** Light microscopic images of rat liver and kidney tissues stained with Masson trichrome (MT). Increased collagen deposition indicative of fibrosis (green arrow) was observed in the liver tissue (Nikon Eclipse Si, Tokyo, Japan. X200 and X400)

*DSF: Disulfiram, EtOH: Ethano*

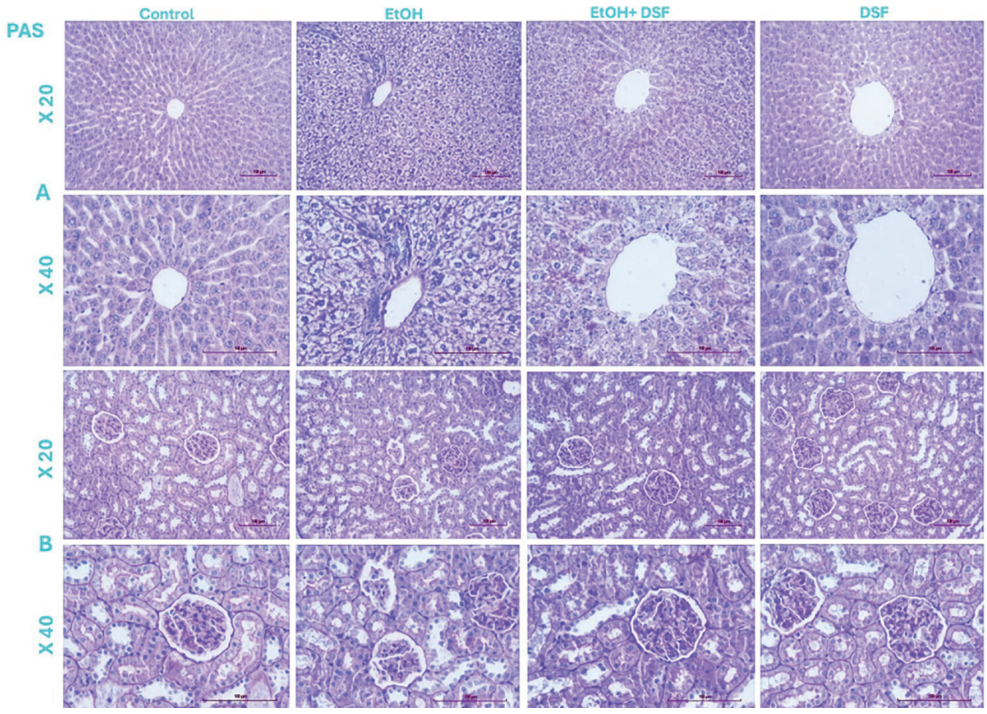


**Figure 4.** Light microscopic images of rat liver and kidney tissues stained with periodic acid-schiff (PAS), (Nikon Eclipse Si, Tokyo, Japan. X200 and X400)

*DSF: Disulfiram, EtOH: Ethanol*

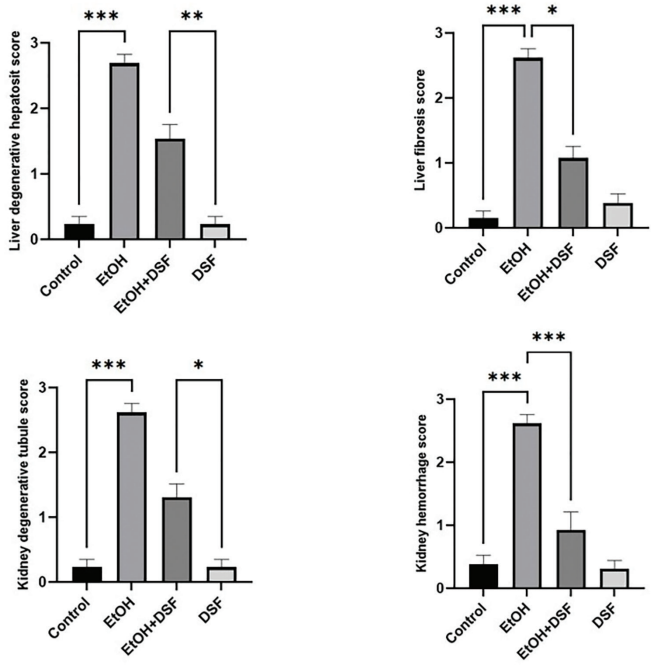


**Figure 5.** Histopathological findings of rat liver and kidney tissues. Graph exhibiting hepatocyte degeneration, fibrosis, tubular degeneration and hemorrhage in the experimental groups. Data were presented as mean ± standard deviation and median (min-max)

**: p<0.05, **: p<0.01, ***: p<0.001, DSF: Disulfiram, EtOH: Ethanol*

**Table 2. Histological scoring of rat liver and kidney tissues showing hepatocyte degeneration, fibrosis, tubular degeneration, and hemorrhage in the experimental groups**

| Liver | Control | EtOH | EtOH + DSF | DSF | p |
|---|---|---|---|---|---|
| Hepatocyte degeneration | 0.000 (0.000-1.000) | 3.000 (2.000-3.000) | 1.000 (1.000-3.000) | 0.000 (0.000-1.000) | <0.001 |
| Fibrosis | 0.000 (0.000-1.000) | 3.000 (2.000-3.000) | 1.000 (0.000-2.000) | 0.000 (0.000-1.000) | <0.001 |
| **Kidney** | | | | | |
| Tubular degeneration | 0.000 (0.000-1.000) | 3.000 (2.000-3.000) | 1.000 (0.000-3.000) | 0.000 (0.000-1.000) | <0.001 |
| Hemorrhage | 0.000 (0.000-1.000) | 3.000 (2.000-3.000) | 1.000 (0.000-3.000) | 0.000 (0.000-1.000) | <0.001 |

Data are presented as mean ± standard deviation and median (min-max). p<0.05, DSF: Disulfiram, EtOH: Ethanol

additionally noted during vacuolization, especially in the proximal tubules (p<0.001). In this group, there was also hemorrhage in the kidney tissue (p<0.001) (Figure 2B). In contrast to liver tissue, no fibrosis was found in the kidney on MT staining (Figure 3B). PAS staining reveals abnormalities in the glomerular basement membranes and a decrease in tubular damage and preservation of brush border architecture (Figure 4B). The glomerular structures in the EtOH+DSF group treated with DSF are relatively preserved, and the basement membrane is clearly visible. Furthermore, it was found that the protective action of DSF reduced tubular damage and mostly preserved the brush-like edge structure. Hemorrhage was markedly reduced in this group throughout the tissue (p<0.001) (Figures 2-5, Table 2).

### Immunohistochemical Results of Liver and Kidney Tissues

#### Liver Assessment

In the control group, IL-1β expression in liver tissue was minimal and weak immunoreactivity was observed around the vessels. Similarly, in the DSF group, IL-1β staining was very weak and did not indicate inflammatory activity. IL-1β density and prevalence were significantly higher in the EtOH group compared to the control group (p<0.001). This marker showed reactivity around the vessels in the tissue. IL-1β expression was considerably lower in the EtOH +DSF group than in the EtOH group (p<0.001) (Figure 6A, Table 3).

#### Kidney Assessment

The expression of IL-1β in kidney tissue was low in both the control and DSF groups. IL-1β expression was significantly elevated in the EtOH group, particularly in proximal tubular cells. When compared to the control group, this rise was statistically significant (p<0.001). In the EtOH + DSF group, IL-1β expression was significantly lower than in the EtOH group (p<0.01) (Figure 6B), with tubular locations showing reduced staining intensity (Table 3).

## Discussion

Acute toxic effects of EtOH lead to pronounced histopathological and immunohistochemical alterations, particularly in metabolically active organs such as the liver and kidneys. In the present study, EtOH administration resulted in marked structural disorganization, cellular damage, and enhanced inflammatory responses in both tissues. DSF treatment, on the other hand, was associated with alleviation of these pathological alterations, potentially related to modulation of IL-1β-mediated inflammatory responses. Carmiel-Haggai et al. (16) treated genetically obese and thin rats with 4 g/kg EtOH every 12 hours for three days. This therapy resulted in a 6.4% loss in body weight in thin rats. According to this study, acute EtOH consumption significantly affects body weight (16). Yoladi et al. (17) reported that rats treated with EtOH had a statistically important decrease in body weight. This conclusion indicates that EtOH has a detrimental effect on food intake and energy balance by altering metabolic processes during the experiment (17). Consistent with our study, in a recent study, rats were given a single injection of EtOH at a dose of 3 g/kg and it was observed that this high dose caused a significant decrease in body weight by suppressing appetite (18). In a study, 6-month-old male Wistar rats were given EtOH in drinking water at a volumetric concentration of 20% for 30 days. Histopathological examination of the liver revealed significant shrinking of the sinusoids, disruption of hepatocyte cords, and complete loss of sinusoidal architecture in some areas. Hepatocytes demonstrated significant vacuolization and degenerative alterations. Renal tissue investigation revealed a considerable reduction in Bowman's space, massive vacuolization in tubular epithelial cells, luminal displacement of nuclei and hydropic degeneration in certain tubular cell nuclei (5). In another study, an acute toxicity model was established by giving 5 g/kg EtOH orally to rats consistent with our dosing protocol. When the liver tissue was examined, ballooning degeneration in hepatocytes, vacuolization in the cytoplasm and also sinusoidal enlargement were observed (19).

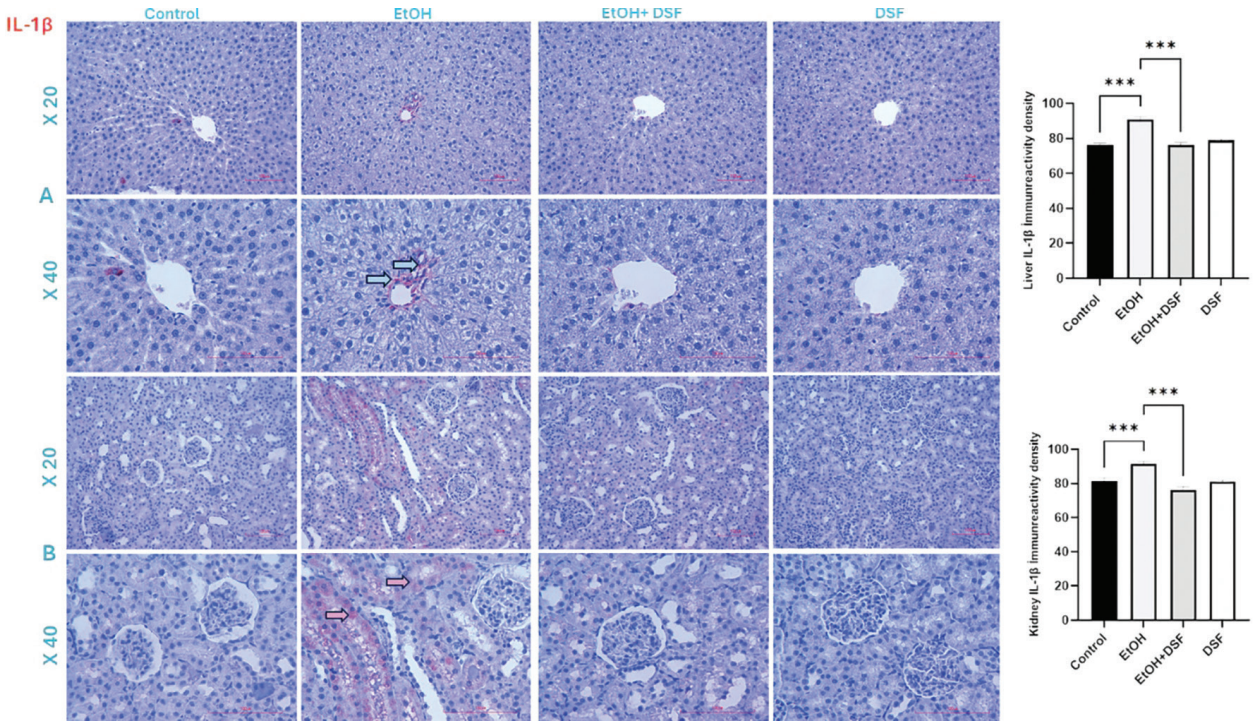


**Figure 6.** Immunohistochemical microscopic images and quantitative analysis of interleukin-1beta (IL-1β) expression in liver and kidney tissues of all experimental groups. Red areas indicate immunopositive staining. Increased IL-1β-positive immunoreactivity in the central vein and surrounding pericentral regions of the liver tissue (blue arrows) and in tubular epithelial cells in kidney tissue, with prominent inflammatory changes (pink arrows). The slides had been counterstained with hematoxylin. Data were presented as mean ± standard deviation and median (min-max), (Nikon Eclipse Si, Tokyo, Japan. X200 and X400)

**: p<0.05, **: p<0.01, ***: p<0.001, DSF: Disulfiram, EtOH: Ethanol*

**Table 3. Immunoreactivity intensity of the IL-1β marker in the experimental groups**

| Liver | Control | EtOH | EtOH + DSF | DSF | p |
|---|---|---|---|---|---|
| IL-1β immunreactivity density | 76.25±3.353 | 90.88±5.251 | 76.27±5.044 | 78.87±2.784 | <0.001 |
| **Kidney** | | | | | |
| IL-1β immunreactivity density | 81.6±5.758 | 91.65±4.528 | 76.27±6.908 | 81.18±3.221 | <0.001 |

Data are presented as mean ± standard deviation and median (min-max)
DSF: Disulfiram, EtOH: Ethanol, IL-1β: Interleukin-1beta

These findings were consistent with our findings. These pathologies are associated with oxidative stress at the cellular level caused by toxic intermediates such as ROS and acetaldehyde released as a result of EtOH metabolism. In addition, inflammatory cell infiltration and the presence of necrotic cells were reported (20). In a study, it was reported that IL-1β expression increased in the central amygdala of mice after chronic EtOH exposure and this increase was confirmed by immunohistochemistry. In this study, the expression of IL-1β in microglia and neurons was evaluated by confocal microscopy (21). In another study, mice were injected with lipopolysaccharide (LPS) 4 hours after oral administration of a single dose of 3 g/kg EtOH. The results showed that EtOH pretreatment significantly increased the mRNA and protein levels of proinflammatory cytokines such as IL-1β, and IL-6 induced by LPS (22). In one study, researchers administered an acute single dose of 5 g/kg EtOH intragastrically and 9 hours later the animals were sacrificed and tissues were taken. They showed that EtOH exposure increased IL-1β and inflammatory response in the liver (23). These findings were consistent with our results regarding IL-1β. Our immunohistochemistry investigations revealed that IL-1β expression was markedly elevated in liver and kidney tissues, suggesting an association between IL-1β immunoreactivity and histopathological findings. Beyond its conventional application, DSF's regulatory function on indicators linked to inflammation and oxidative stress has been investigated in several experimental animal studies (24-26).

In a study investigating the hepatoprotective effects of DSF, DSF treatment was shown to significantly reduce fibrotic changes in experimental liver fibrosis models induced by carbon tetrachloride administration and bile duct ligation in rats (27). Lei et al. (28) showed that DSF treatment significantly reduced liver steatosis and fibrosis in a non-alcoholic steatohepatitis (NASH) model and suppressed inflammation and oxidative stress. Zhang et al. (29) used DSF therapy in a rat model of renal fibrosis caused by unilateral ureteral obstruction. DSF therapy was found to significantly reduce the accumulation of collagen and the expression of fibrotic markers in histopathological examination. Furthermore, the findings demonstrated that DSF inhibited the gasdermin D protein, which in turn decreased inflammation and pyroptosis (29). Another recent study reported that NLRP3 inflammasome activity was inhibited by DSF. This was supported by decreased expression levels of NLRP3, caspase-1 (p20) and IL-1β proteins (30). A model of fungal keratitis caused by Aspergillus fumigatus was used to assess the effects of DSF. According to the study, DSF therapy blocked IL-1β release, reduced neutrophil and macrophage infiltration into ocular tissue, and regulated the inflammatory response. These results imply that DSF may lessen inflammation and thereby minimize tissue damage (31). In another study, the effects of DSF were examined in a peritendinous fibrosis model that developed after tendon injury in mice. In this study, it was shown that DSF treatment significantly decreased the levels of proinflammatory cytokines such as IL-1β and IL-1α prevented the development of fibrosis (32). All these results were in parallel with our results in terms of IL-1β. DSF decreased IL-1β reactivity, which was increased in liver and kidney in our study. Immunohistochemical analysis showed that IL-1β expression was markedly increased in the EtOH group. These findings suggest that IL-1β may play an important role in the acute inflammatory response under the present experimental conditions. DSF treatment was associated with reduced IL-1β immunoreactivity in liver and kidney tissues. Numerous studies have previously demonstrated that DSF modulates inflammatory processes (33-35). DSF has been highlighted for its ability to prevent tissue damage by inhibiting the NLRP3 inflammasome and attenuating oxidative stress. The results of the present study further support the anti-inflammatory properties of DSF against EtOH-induced acute toxicity (36).

### Study Limitations

Despite these promising findings, certain limitations should be acknowledged. In the present study, IL-1β was evaluated as the primary inflammatory marker, while other relevant cytokines were not assessed, which may limit a more comprehensive understanding of the inflammatory response. Additionally, the absence of complementary quantitative analyses, such as Western blotting or ELISA, to validate the immunohistochemical findings may restrict the precision of molecular-level interpretations.

## Conclusion

In summary, our investigation showed that acute EtOH intake significantly damaged the liver and kidneys both structurally and through inflammatory mechanisms, a process that was primarily linked to elevated IL-1β levels. By lowering histopathological degradation and inhibiting the inflammatory process, particularly IL-1β-mediated inflammation, DSF therapy demonstrated a significant protective effect. These findings suggest that DSF may represent a potential candidate for further experimental investigation in the modulation of alcohol-induced inflammatory organ damage, in addition to its established use in the treatment of alcohol dependence. Future studies should thoroughly examine DSF's optimal dosage range, molecular mechanisms of action, and potential synergistic effects with other anti-inflammatory drugs.

### Ethics

**Ethics Committee Approval:** All animal procedures were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council). This study was reviewed by the Local Ethics Committee of Kırşehir Ahi Evran University Animal Experiments and this committee approved our experimental guidelines (decision no: 24/057, date: 19.01.2024).

**Informed Consent:** N/A.

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### Footnotes

### Authorship Contributions

Surgical and Medical Practices: K.T.K., S.K., E.K., N.Ç., H.T.Y., Concept: K.T.K., Design: K.T.K., S.K., N.Ç., B.Y., Data Collection or Processing: K.T.K., S.K., E.K., N.Ç., B.Y.,

H.T.Y., Analysis or Interpretation: K.T.K., E.K., B.Y., H.T.Y., Literature Search: K.T.K., Writing: K.T.K.

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