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BASIC MEDICAL SCIENCES / TEMEL TIP BİLİMLERİ

# Immunoreactivity of NOS2 and NF-κB in Kidney Tissue in Experimental Alcohol Consumption Model

## Deneysel Alkol Tüketimi Modelinde Böbrekteki NOS2 ve NF-κB'nin İmmünreaktivitesi

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## Abstract

**Objectives:** The negative effects of alcohol on health have attracted attention in recent years. The most devastating complications of alcoholism, such as kidney damage, can be seen due to the continuous consumption of alcohol. Possible mechanisms by which alcohol may increase renal dysfunction have been expressed in the literature. Among these mechanisms, oxidative stress is thought to be a potential mechanism that affects kidney function. Nitric oxide synthase (NOS) and nuclear factor kappa B (NF-κB) levels, which have roles in oxidative stress and inflammation, may be at abnormal levels in the kidney in alcohol use disorder. This study aimed to evaluate the role of NOS and NF-κB molecules in the mechanism of kidney damage caused by alcohol use.

**Materials and Methods:** The immunoreactivity of NOS2 and NF-κB in kidney tissue was evaluated in an experimental model of acute and chronic alcohol intake in male and female rats (n=56). Groups, control female, control male, sham female, sham male, acute male model, acute female model, chronic female model and chronic male model. The acute and chronic model groups were given ethanol to induce alcohol intake. Immunohistochemical analyzes were performed for NOS2 and NF-κB expressions along with histopathological analysis in kidney tissues.

**Results:** It was observed that glomerulation degeneration, bleeding, vacuolization, and inflammation were increased in kidney tissues in all groups compared to control groups. In addition, NF-κB and NOS2 expressions were found to be significantly higher in the acute and chronic model groups compared to the control groups.

**Conclusion:** The presented findings reveal that the expression of NOS2, which is involved in oxidative stress, and NF-κB, which is involved in inflammation, increases kidney damage in acute and chronic alcohol intake. Therefore, NFκB and NOS2 proteins, which play a role in tissue damage, inflammation, and oxidative stress response, may be associated with alcohol-induced renal damage.

**Key Words:** Alcohol Consumption Model, NF-κB, NOS2, Kidney, Oxidative Stress

## Öz

**Amaç:** Alkolün sağlık üzerindeki olumsuz etkileri son yıllarda dikkatleri üzerine çekmektedir. Kronik alkol tüketimi nedeniyle böbrek hasarı gibi yıkıcı komplikasyonlar görülebilir. Alkolün böbrek fonksiyon bozukluğunu artırabileceği olası mekanizmalar literatürde ifade edilmiştir. Bu mekanizmalar arasında oksidatif stresin böbrek fonksiyonlarını etkileyen potansiyel bir mekanizma olduğu düşünülmektedir. Oksidatif stres ve enflamasyonda rolü olan nitrik oksit sentaz (NOS) ve nükleer faktör kappa B (NF-κB) seviyeleri, alkol kullanım bozukluğunda böbrekte anormal düzeylerde görülebilmektedir. Bu çalışmada alkol kullanımına bağlı böbrek hasarının mekanizmasında NOS ve NF-κB moleküllerinin rolünün değerlendirilmesi amaçlanmıştır.

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## Öz

**Gereç ve Yöntem:** Böbrek dokusunda NOS2 ve NF-κB'nin immünoreaktivitesi, erkek ve dişi sıçanlarda (n=56) deneysel bir akut ve kronik alkol alımı modelinde değerlendirildi. Gruplar, kontrol dişi, kontrol erkek, sham dişi, sham erkek, akut erkek model, akut dişi model, kronik dişi model ve kronik erkek model olarak ayrılmıştır. Akut ve kronik model gruplarına alkol alımını teşvik etmek için etanol verildi. Böbrek dokularında histopatolojik analiz ile birlikte NOS2 ve NF-κB ekspresyonları için immünohistokimyasal analizler yapıldı.

**Bulgular:** Kontrol grubuna göre tüm gruplarda böbrek dokularında glomerülasyon dejenerasyonu, kanama, vakuolizasyon ve enflamasyonun arttığı görüldü. Ayrıca NF-κB ve NOS2 ekspresyonları akut ve kronik model gruplarında kontrol gruplarına göre anlamlı olarak yüksek bulunmuştur.

**Sonuç:** Sunulan bulgular, akut ve kronik alkol alımında oksidatif streste yer alan NOS2 ve enflamasyonda rol oynayan NF-κB ekspresyonunun böbrek hasarını artırdığını ortaya koymaktadır. Bu nedenle, doku hasarı, enflamasyon ve oksidatif stres yanıtında rol oynayan NF-κB ve NOS2 proteinleri, alkolün neden olduğu böbrek hasarı ile ilişkili olabilir.


**Anahtar Kelimeler:** Alkol Tüketim Modeli, NF-κB, NOS2, Böbrek, Oksidatif Stres

## Introduction

Alcohol consumption is one of the leading causes of preventable deaths worldwide, with 3 million deaths per year attributable to alcohol. Alcohol-related morbidity and mortality are largely due to the high rates of alcohol use disorder in the population (1). Chronic alcohol consumption can often be associated with clinical diseases such as breast and colon cancer, pancreatic disease, liver cirrhosis, diabetes, osteoporosis, arthritis, kidney disease, immune system dysfunction, and hypertension (2). In addition, a relationship between kidney diseases due to alcohol use disorder has been shown in the literature (3).

Studies in the literature report that the incidence of kidney disease is lower in heavy alcohol drinkers (more than 210 g/week alcohol consumption) compared to moderate alcohol drinkers (70-210 g/week alcohol consumption) (4,5). The possible relationship between alcohol consumption and kidney damage has not yet been fully elucidated. In the literature, it has been suggested that oxidative stress resulting from increased production of reactive oxygen species (ROS) may play a role in pathogenesis. Increased ROS can trigger excessive amounts of free radicals, triggering tissue damage in the kidney. In addition, the effect on other major organs (liver, heart, intestines and skeletal muscle) in alcohol use disorder can trigger adverse pathological processes that damage the kidneys (3).

A clinical study conducted in 2018 showed its association with alcohol use disorder due to the development of chronic kidney disease. An approximately two-fold increase in the incidence of newly diagnosed chronic kidney disease has been reported in alcohol use disorder (6). Alcohol addiction may be a risk factor for the development of chronic kidney disease, especially in the younger population. A study in 2022 also investigated the mortality rate, disease progression, and disease burden of acute kidney injury in the alcohol use disorder population. It was stated that the subpopulation of patients with alcohol use disorder had a higher number of patients with acute kidney injury and a higher mortality rate than the subpopulation without alcohol use disorder (7). Therefore, determining the pathogenesis of kidney damage in individuals with alcohol use disorder may be important in preventing alcohol-related mortality.

Nitric oxide synthase (NOS), a molecule associated with oxidative stress, may be involved in alcohol dependence (8). Nitric oxide (NO), the smallest known signaling molecule, is produced by three isoforms of NOS. All use l-arginine and molecular oxygen as substrates (9). The NO pathway is involved in the regulation of glomerular hemodynamics. The net effect in the kidney is to support natriuresis and diuresis, to adapt to changes in dietary salt intake, and to contribute to the maintenance of normal blood pressure (10). In a study, the effect of the NOS cofactor tetrahydrobiopterin during chronic alcohol consumption was investigated. Sprague-Dawley rats fed alcoholic or non-alcoholic liquid diets for 2-3 months have demonstrated impaired NOS-induced vasodilation during alcohol consumption (11). In this context, NOS in chronic alcohol consumption may affect kidney damage about oxidative stress.

NF-κB, which is involved in tissue damage and inflammatory processes, are inducible transcription factors (12). NF-κB stimulates two major signaling pathways, canonical and non-canonical (or alternative) that are responsible for regulating immune and inflammatory responses (13). The most important function of NF-κB is the regulation of inflammatory responses. NF-κB, mediates the induction of various proinflammatory genes in innate immune cells (14). Activation of the NF-κB signaling pathway is associated with the expression of NOS and cyclooxygenase-2 enzymatic proteins (15,16). In this context, NF-κB may act by triggering ROS and oxidative stress in alcohol dependence. In a study conducted in 2020, an agent trial was conducted that affects the NF-κB signaling pathway in chronic alcoholic liver disease. It has been shown that andrographolide can reduce alcohol-induced liver pathological damage and oxidative stress by reducing the expression of NF-κB and TNF-α in mice exposed to ethanol (17). Thus, the NOS-mediated NF-κB signaling pathway may be activated in the liver and kidney in alcohol-induced tissue injury.

In this study, the immunoreactivity of NOS and NF-κB molecules was investigated for the mechanism of kidney damage in alcohol dependence. NOS and NF-κB expressions were determined by immunohistochemistry in rats with alcohol dependence through ethanol. In addition, the pathology of kidney damage in alcohol dependence was evaluated by H&E staining.

## Materials and Methods

### Experimental Groups

This study was carried out with ethical approval from Erciyes University Animal Experiments Local Ethics Committee (HAYDEK) (approval no: 21/059, date: 07.04.2021). Experimental groups were designed as follows.

**Control Group (n=7):** Animals in this group were not treated.

**Sham Group (n=7):** Animals in this group were given only the volume (2 mL) of distilled water given to the animals in the acute model and chronic model by oral gavage.

**Acute Model Group (n=7):** Animals in this group were given 18% v/v ethanol (prepared in distilled water) in a total volume (2 mL) and a total dose of 1 g/kg body weight by oral gavage (18). It is planned to have at least two days between two gavage applications. In other words, it is aimed to create a model of alcohol exposure for a period of approximately two weeks in total. Thus, it was intended to represent a consumption habit reported in the majority of rats, typically referred to as acute, low-moderate alcohol intake (19,20).

**Chronic Model Group (n=7):** Animals in this group were administered 20% v/v ethanol (prepared in distilled water) in a total volume (2 mL) by oral gavage at a total dose of 4.5 g/kg body weight (21).

Gavage was applied to the rats every day between 9.00-10.00 in the morning (daylight). Body weights were measured every week and it was observed whether they gained weight or lost weight. No restrictions were applied on feed and water consumption. After the experimental protocol was completed, the experimental animals were decapitated and the kidney tissue was taken.

### Histological Analysis

After 10% formaldehyde fixation, kidney tissue samples were cleared in xylene and embedded in paraffin after passing through increasing alcohol series (70%, 80%, 96%, and 100%). Sections were taken as 5 µm for hematoxylin and eosin (H&E) staining and immunohistochemical staining (22). It was examined under a light microscope at different magnifications. Lesions in glomerular degeneration and tubulointerstitial injury (such as vacuolization, bleeding, and infiltration of inflammatory cells) were evaluated separately for each animal and were graded from 0-3: 0 absent, 1 mild, 2 moderate, and 3 severe in each category. Quantification was performed on 30 glomeruli randomly selected by two investigators and randomly selected cortical areas per 20 sections, and statistical analysis was performed on mean values from each animal group, n=7 rats per group (23).

### Immunohistochemical Analysis

Immunoreactivities of NF-κB (BT-MCA1291, Bioassay Technology Laboratory) and NOS2 (E-AB-70051, Elabscince) proteins were determined by immunohistochemical analysis in sections taken from kidney tissues of experimental groups using the Avidin-Biotin peroxidase method (22). In summary, after deparaffinization of 5µm thick sections, citrate buffer was used to open epitopes (pH: 6.0; Thermo Fischer Scientific, UK, AP-9003-500). Then slides were placed in a 3% hydrogen peroxide solution in methanol to inhibit endogenous peroxidase activity. Ultra V block solution (Thermo Fischer Scientific, UK, TA-125-UB) was applied to prevent non-specific staining. Then, it was incubated with primary antibodies (NF-kB was used at dilution ratios of 1:50 and NOS2 at 1:300 dilution ratios) overnight at 4 °C. After, it was incubated with biotinylated goat anti-polyvalent secondary antibody (Thermo Fisher Scientific, UK, TP-125-BN) for 40 minutes in a 37 °C oven. After washing several times with PBS, it was incubated with streptavidin peroxidase (Thermo Fisher Scientific, UK, TS-125-HR) for 30 minutes in a 37 °C oven. The antibody complex was visualized by incubation with diaminobenzidine (DAB) chromogen (Thermo Fisher Scientific, UK, TA-125-HD). Sections were then counterstained with Gill III Hematoxylin (Merck, Germany, 1.05174.1000). It was dehydrated by passing through a series of increasing alcohol and covered with a sealant called entellan. Sections were examined with an Olympus BX53 light microscope. Evaluation of immunoreactivity levels was done with ImageJ Version 1.46 (National Institutes of Health, Bethesda, Maryland).

### Statistical Analysis

GraphPad Prism 8.0 software program was used for all statistical analysis and graph drawing. Two-way ANOVA analysis of variance and Tukey's multiple comparison tests were applied to analyze the numerical data obtained from the observed damage rates in the kidney. $P<0.05$ was considered statistically significant.

## Results

### Histological Results

It was observed that glomerulation degeneration, bleeding, vacuolization, and inflammation were increased in kidney tissues in acute and chronic models of both sexes compared to control groups (Figure 1, Table 1). Especially in the acute male

group, glomerular degeneration, bleeding, and vacuolization were found to be significantly increased compared to the acute female group (**p<0.001, *p<0.05; Table 1). While there was a significant decrease in vacuolization in the chronic male group compared to the chronic female group, an increase in inflammation was observed (***p<0.0001; Table 1).

### Immunohistochemical Results

NF-κB and NOS2 expressions were significantly higher in the acute and chronic model groups compared to the control groups (Figure 2). While NF-κB expression increased 3.6 times in the acute female group and 4.9 times in the chronic female group compared to the control female group, it was observed that it increased 4.2 times in the acute male group and 4.4 times in the chronic male group compared to the control male group. While the NF-κB expression observed in the chronic female group was found to be significantly higher than in the acute female group, no difference was observed between the acute male and chronic male groups.

While NOS2 expression increased 3.08-fold in the acute female group and 3.1-fold in the chronic female group compared to the control female group, it was observed that it increased 3.6-fold in the acute male group and 4.3-fold in the chronic male group compared to the control male group. It was determined that there was no difference between the acute and chronic model groups.

When the female and male acute models were compared within themselves, no difference was observed in terms of NF-κB and NOS2 expression. While NOS2 expression increased significantly in the chronic male group compared to the chronic female group, there was no difference in NF-κB expression.

## Discussion

In recent years, the negative effects of alcohol on health have reached remarkable dimensions. Mortality and morbidity rates resulting from alcohol consumption are increasing every year. Complications such as kidney damage and liver failure can often be seen due to the continuous consumption of alcohol. Chronic alcohol consumption can seriously affect kidney damage. In our study, we found that the immunoreactivity of NF-κB and NOS2 in the kidney was increased in both acute and chronic alcohol intake models compared to control groups. While the NF-κB expression observed in the chronic female group was significantly higher than in the acute female group, no difference was observed between the acute male and chronic

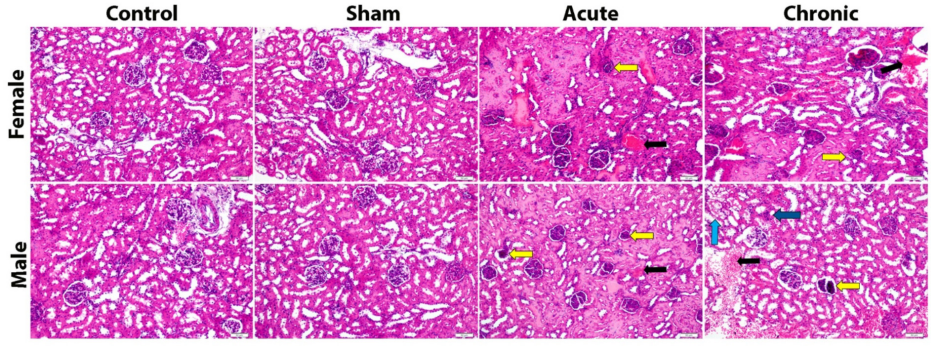


**Figure 1:** H&E staining images of kidney tissues of the experimental groups. The black arrow indicates hemorrhage, the yellow arrow indicates glomerular degeneration, the light blue arrow vacuolization, and the dark blue arrow inflammation. Magnification 20X, scale bar= 50 µm

**Table 1: Kidney damage rates by groups**

| | | Tubulointerstitial lesions | | |
|---|---|---|---|---|
| **Groups** | **Glomerular degeneration** | **Hemorrhage** | **Vacuolization** | **Inflammation** |
| Control female | 0.38±0.05 | 0.34±0.05 | 0.44±0.06 | 0.20±0.05 |
| Sham female | 0.36±0.04 | 0.31±0.05 | 0.50±0.08 | 0.25±0.03 |
| Acute female | 1.21±0.12$^{ab}$ | 1.40±0.32$^{ab}$ | 1.06±0.18$^{ab}$ | 0.73±0.04$^{ab}$ |
| Chronic female | 1.98±0.18$^{abc}$ | 2.01±0.26$^{abc}$ | 2.09±0.39$^{abc}$ | 0.80±0.07$^{ab}$ |
| Control male | 0.40±0.07 | 0.35±0.14 | 1.10±0.22 | 0.33±0.08 |
| Sham male | 0.41±0.06 | 0.23±0.08 | 0.40±0.04 | 0.37±0.18 |
| Acute male | 1.62±0.16$^{de**}$ | 1.84±0.31$^{de**}$ | 1.42±0.29$^{de*}$ | 1.08±0.30$^{de}$ |
| Chronic male | 2.20±0.13$^{def}$ | 2.06±0.19$^{de}$ | 1.55±0.40$^{de***}$ | 1.49±0.30$^{def***}$ |

Two-way analysis of variance and Tukey's multiple comparison tests were applied. Data shown in the table are expressed as mean ± standard deviation. $^{a}$: p<0.05 compared to the control female group; $^{b}$: p<0.05 to the sham female group; $^{c}$: p<0.05 compared to the acute female group; $^{d}$: p<0.05 compared to the control male group; $^{e}$: p<0.05 compared to the sham male group; $^{f}$: p<0.05 shows that there is a statistical difference compared to the acute male group, *p<0.05, **p<0.001, ***p<0.0001

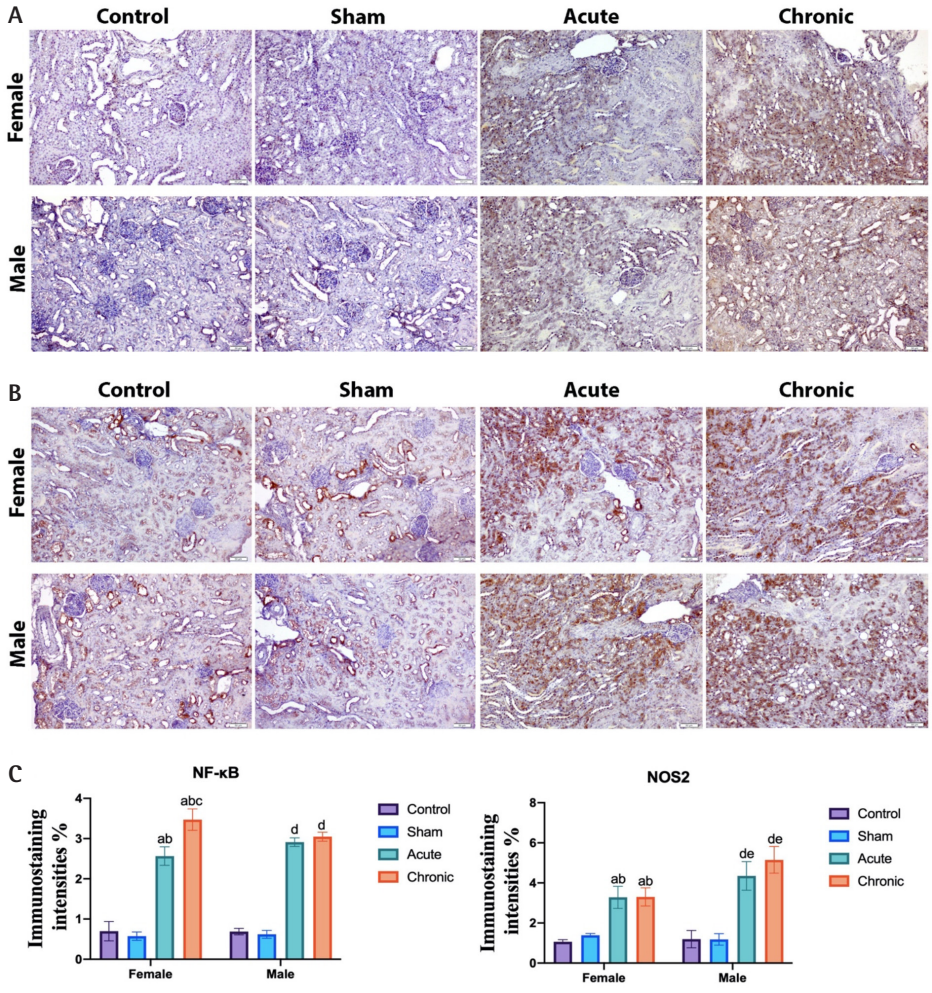


**Figure 2.** NF-κB and NOS2 immunostaining images in the kidney tissues of the experimental groups. A) NF-κB immunostaining images. B) NOS2 immunostaining images. Magnification 20X, scale bar=50 µm. C) Bar graphs showing immunostaining intensities. Data shown in bar graphs are expressed as mean ± standard deviation. Two-way analysis of variance and Tukey's multiple comparison tests were applied. a: means p<0.05 from the control female group, b: p<0.05 from the sham female group, c: p<0.05 from the acute female group, d: p<0.05 from the control male group, e: from the p<0.05 sham male group, and f: p<0.05 from the acute male group

male groups. When the female and male acute models were compared within themselves, no difference was observed in terms of NF-κB and NOS2 expression. While NOS2 expression increased significantly in the chronic male group compared to the chronic female group, there was no difference in NF-κB expression. Therefore, NF-κB and NOS2 proteins, which play a role in tissue damage, inflammation, and oxidative stress response, may be associated with alcohol-induced renal damage.

Chronic alcohol consumption can affect kidney damage to a certain extent. A 2017 study showed that acute alcohol poisoning exacerbated acute renal failure due to rhabdomyolysis in rats. The renal tissue injury parameters NF-κB and inducible NOS (iNOS) were evaluated by giving rats intravenous injections of 5 g/kg ethanol for 3 hours (24). In this context, acute alcohol intoxication may exacerbate renal failure through pro-oxidant and inflammatory effects such as iNOS and NF-κB. In correlation with our results, NF-κB and NOS2 expression increases due to damage to the mechanism of alcoholic nephropathy. In addition, new studies on kidney damage due to acute and chronic alcohol intake are needed in the literature.

iNOS mediates ethanol-induced redox imbalance and upregulation of inflammatory cytokines in the kidney. Renal damage was investigated by administering ethanol (20% v/v) to C57BL/6 wild-type and iNOS gene-deficient (iNOS-/-) mice for 10 weeks (25). iNOS may play a role in ethanol-induced oxidative stress and proinflammatory cytokine production in the kidney. In another study, it was determined that inhibition of NF-κB reduced glycerol-induced kidney damage. Wistar rats were given 8 ml/kg of 50% glycerol intramuscularly and their renal parameters were evaluated (26). In the context of these data, decreased NF-κB expression may reduce alcohol-induced kidney damage.

In the literature, the role of CYP2E1 in the regulation of NOS and NF-κB is emphasized. Therefore, a 2015 study observed that CYP2E1 was induced in the renal tubules of mice on a chronic alcohol diet (27). Therefore, it can be stated that kidney damage due to chronic ethanol intake can be induced by CYP2E1 in the liver. In addition, induction of CYP2E1 can lead to the generation of ROS and oxidative stress (28). NOS, a molecule involved in the oxidative stress mechanism and shown to be induced by CYP2E1 (29), has been reported to stimulate the production of proinflammatory cytokines through the activation of redox-sensitive NF-κB by exerting the pathological effects of CYP2E1 in alcohol damage by increasing nitroxidative stress and lipid peroxidation (30). Therefore, enzymes such as CYP2E1 may have a role in the mechanism that triggers the activation of NOS2 and NF-κB in acute and chronic alcohol consumption-induced kidney damage. Determination of molecules that trigger oxidative stress and inflammatory processes in renal damage due to alcohol dependence is considered important in pathogenesis.

### Study Limitations

Our data show increased expression of NOS2 and NF-κB in acute and chronic alcohol consumption-induced kidney damage. However, only the demonstration of immunohistochemical changes can be considered as the limitation of the study. In future studies, a comprehensive characterization can be revealed by investigating the protein and mRNA expressions of NOS2, NF-κB and other inflammatory mediators in the same tissues.

## Conclusion

Our data show increased expression of NOS2 and NF-κB in the kidney in the acute and chronic alcohol intake model induced in rats. On the other hand, only the demonstration of immunohistochemical changes can be considered a limitation of our study. In future studies, a comprehensive characterization can be revealed by examining the protein and mRNA expressions of NOS2, NF-κB, and other inflammation and oxidative stress parameters in similar tissues.

### Ethics

**Ethics Committee Approval:** This study was carried out with ethical approval from Erciyes University Animal Experiments Local Ethics Committee (HAYDEK) (approval no: 21/059, date: 07.04.2021).

**Informed Consent:** Animal experiment study.

**Peer-reviewed:** Externally peer-reviewed.

### Authorship Contributions

Surgical and Medical Practices: A.C.U., S.Y., Z.D., Concept: A.O., A.C.U., O.Ö., S.Y., Z.D., Design: A.O., A.C.U., S.Y., Z.D., Data Collection and Processing: A.O., A.C.U., O.Ö., E.E., S.Y., Z.D., Analysis or Interpretation: A.O., A.C.U., O.Ö., S.Y., Z.D., Literature Search: A.O., A.C.U., O.Ö., E.E., S.Y., Z.D., Writing: A.O., A.C.U., O.Ö., E.E., S.Y., Z.D.

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## References

1. Witkiewitz K, Litten R, Leggio L. Advances in the science and treatment of alcohol use disorder. Sci Adv. 2019;5:eaax4043.
2. Dguzeh U, Haddad NC, Smith KTS, et al. Alcoholism: A Multi-Systemic Cellular Insult to Organs. Int J Environ Res Public Health. 201828;15:1083.
3. Varga ZV, Matyas C, Paloczi J, et al. Alcohol Misuse and Kidney Injury: Epidemiological Evidence and Potential Mechanisms. Alcohol Res. 2017;38:283-288.
4. Reynolds K, Gu D, Chen J, et al. Alcohol consumption and the risk of end-stage renal disease among Chinese men. Kidney Int. 2008;73:870-876.
5. Buja A, Scafato E, Baggio B, et al. Renal impairment and moderate alcohol consumption in the elderly. Results from the Italian Longitudinal Study on Aging (ILSA). Public Health Nutr. 2011;14:1907-1918.
6. Pan CS, Ju TR, Lee CC, et al. Alcohol use disorder tied to development of chronic kidney disease: A nationwide database analysis. PLoS One. 2018;13:e0203410.
7. Maharjan J, Le S, Green-Saxena A, et al. Mortality, disease progression, and disease burden of acute kidney injury in alcohol use disorder subpopulation. Am J Med Sci. 2022;364:46-52.
8. Bagyánszki M, Krecsmarik M, De Winter BY, et al. Chronic alcohol consumption affects gastrointestinal motility and reduces the proportion of neuronal NOS-immunoreactive myenteric neurons in the murine jejunum. Anat Rec (Hoboken). 2010;293:1536-1542.
9. Förstermann U, Sessa WC. Nitric oxide synthases: regulation and function. Eur Heart J. 2012;33:829-837.
10. Lee J. Nitric oxide in the kidney : its physiological role and pathophysiological implications. Electrolyte Blood Press. 2008;6:27-34.
11. Sun H, Patel KP, Mayhan WG. Tetrahydrobiopterin, a cofactor for NOS, improves endothelial dysfunction during chronic alcohol consumption. Am J Physiol Heart Circ Physiol. 2001;281:H1863-1869.
12. Oeckinghaus A, Ghosh S. The NF-kappaB family of transcription factors and its regulation. Cold Spring Harb Perspect Biol. 2009;1:a000034.
13. Sun SC. Non-canonical NF-κB signaling pathway. Cell Res. 2011;21:71-85.
14. Lawrence T. The nuclear factor NF-kappaB pathway in inflammation. Cold Spring Harb Perspect Biol. 2009;1:a001651.
15. Lim JW, Kim H, Kim KH. Nuclear factor-kappaB regulates cyclooxygenase-2 expression and cell proliferation in human gastric cancer cells. Lab Invest. 2001;81:349-360.
16. Arias-Salvatierra D, Silbergeld EK, Acosta-Saavedra LC, et al. Role of nitric oxide produced by iNOS through NF-κB pathway in migration of cerebellar granule neurons induced by Lipopolysaccharide. Cell Signal. 2011;23:425-435.

17. Song Y, Wu X, Yang D, et al. Protective Effect of Andrographolide on Alleviating Chronic Alcoholic Liver Disease in Mice by Inhibiting Nuclear Factor Kappa B and Tumor Necrosis Factor Alpha Activation. J Med Food. 2020;23:409-415.
18. Nguyen TMT, Steane SE, Moritz KM, et al. Prenatal alcohol exposure programmes offspring disease: insulin resistance in adult males in a rat model of acute exposure. J Physiol. 2019;597:5619-5637.
19. McCormack C, Hutchinson D, Burns L, et al. Prenatal Alcohol Consumption Between Conception and Recognition of Pregnancy. Alcohol Clin Exp Res. 2017;41:369-378.
20. Muggli E, O'Leary C, Donath S, et al. "Did you ever drink more?" A detailed description of pregnant women's drinking patterns. BMC Public Health. 2016;16:683.
21. Bonthius DJ, West JR. Alcohol-induced neuronal loss in developing rats: increased brain damage with binge exposure. Alcohol Clin Exp Res. 1990;14:107-18.
22. Doğanyiğit Z, Okan A, Kaymak E, et al. Investigation of protective effects of apilarnil against lipopolysaccharide induced liver injury in rats via TLR 4/ HMGB-1/ NF-κB pathway. Biomed Pharmacother. 2020;125:109967.
23. Inandiklioglu N, Doganyigit Z, Okan A, et al. Nephroprotective effect of apilarnil in lipopolysaccharide-induced sepsis through TLR4/NF-κB signaling pathway. Life Sci. 2021;284:119875.
24. Tsai JP, Lee CJ, Subeq YM, et al. Acute Alcohol Intoxication Exacerbates Rhabdomyolysis-Induced Acute Renal Failure in Rats. Int J Med Sci. 2017;14:680-689.
25. da Silva CBP, Ceron CS, Mendes AS, et al. Inducible nitric oxide synthase (iNOS) mediates ethanol-induced redox imbalance and upregulation of inflammatory cytokines in the kidney. Can J Physiol Pharmacol. 2021;99:1016-1025.
26. de Jesus Soares T, Costa RS, Balbi AP, et al. Inhibition of nuclear factor-kappa B activation reduces glycerol-induced renal injury. J Nephrol. 2006;19:439-448.
27. Latchoumycandane C, Nagy LE, McIntyre TM. Myeloperoxidase formation of PAF receptor ligands induces PAF receptor-dependent kidney injury during ethanol consumption. Free Radic Biol Med. 2015;86:179-190.
28. Gonzalez FJ. Role of cytochromes P450 in chemical toxicity and oxidative stress: studies with CYP2E1. Mutat Res. 2005;569:101-110.
29. Lu Y, Cederbaum AI. CYP2E1 and oxidative liver injury by alcohol. Free Radic Biol Med. 2008;44:723-738.
30. Lu Y, Cederbaum AI. CYP2E1 potentiation of LPS and TNFα-induced hepatotoxicity by mechanisms involving enhanced oxidative and nitrosative stress, activation of MAP kinases, and mitochondrial dysfunction. Genes Nutr. 2010;5:149-167.