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Original Article

# The effect of Anatolian syrup on experimentally induced acetaminophen and lipopolysaccharide associated acute kidney injury

## *Anadolu şurubunun ratlarda deneysel olarak oluşturulan asetoaminofen ve lipopolisakkarit ilişkili akut böbrek hasarı üzerine etkisi*

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## ABSTRACT

**Background:** Acute kidney injury develops as a result of various etiologies and pathological mechanisms, with a high mortality rate. This study aimed to investigate the efficacy of Anatolian syrup on histopathological variables in experimentally induced acetaminophen and lipopolysaccharide associated acute kidney injury.
**Methods:** In this study, 5 groups were formed using 40 male Wistar albino rats (200-220g, 2-4 months old), as follows: Sham, acetaminophen (1 g/kg intraperitoneal (ip)), 3) lipopolysaccharide (5 mg/kg ip), acetaminophen (1 g/kg ip) + Anatolian syrup (15 days orally), and lipopolysaccharide (5 mg/kg ip) + Anatolian syrup (15 days orally).Tubular atrophy, tubular dilatation, cytoplasmic vacuolization in tubular epithelial cells, tubular epithelial cell necrosis, interstitial inflammation, congestion, hemorrhage, glomerular damage and loss of brushy border were evaluated histopathologically semiquantitatively using scoring from 0 to 5.
**Results:** In histopathological variables, tubular atrophy, tubular dilatation, cytoplasmic vacuolization, necrosis, congestion, hemorrhage, glomerular damage, and loss of brushy border were significantly reduced in the acetaminophen + Anatolian syrup group compared to the acetaminophen group and in the lipopolysaccharide + Anatolian syrup group compared to the lipopolysaccharide group ($p<0.001$).
**Conclusions:** The Anatolian syrup was shown to protect histopathological variables in kidney damage caused by acetaminophen and lipopolysaccharide.

**Keywords:** Acute kidney injury, Anatolian syrup, acetaminophen, lipopolysaccharide, histopathology, herbal therapy

## ÖZ

**Giriş ve Amaç:** Akut böbrek hasarı yüksek ölüm oranına sahip olup farklı etiyoloji ve patolojik mekanizmalar sonucunda gelişmektedir. Bu çalışmada Anadolu şurubu'nun deneysel olarak oluşturulan parasetamol ve lipopolisakkarit ilişkili akut böbrek hasarında histopatolojik parametreler üzerindeki etkinliğini araştırmak amaçlanmaktadır.
**Yöntem ve Gereçler:** Bu çalışmada 40 adet Wistar albino cinsi 2-4 aylık 200-220 gr erkek sıçanlar kullanılarak Sham, asetoaminofen (1 g/kg ip), lipopolisakkarit (5 mg/kg ip), asetoaminofen (1 g/kg ip) +Anadolu şurubu (15 gün oral) ve lipopolisakkarit (5 mg/kg ip) + Anadolu şurubu (15 gün oral) olmak üzere 5 grup oluşturulmuştur. Tubuler atrofi, tubuler dilatasyon, tubul epitel hücrelerinde sitoplazmik vakuolizasyon, tubul epitel hücre nekrozu, interstisyel inflamasyon, konjesyon, hemoraji, glomerüler hasar ve fırçamsı kenar kaybı semikantitatif olarak 0'dan 5'e kadar skorlama ile histopatolojik değerlendirilmiştir.
**Bulgular:** Histopatolojik parametrelerde tubuler atrofi, tubuler dilatasyon, sitoplazmik vakuolizasyon, nekroz, konjesyon, hemoraji, glomerüler hasar, fırçamsı kenar kaybı

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açısından asetoaminofen + Anadolu şurubu grubunda asetoaminofen grubuna göre ve lipopolisakkarit + Anadolu şurubu grubunda lipopolisakkarit grubuna göre anlamlı derecede azalma izlenmiştir ($p<0.001$).
**Tartışma ve Sonuç:** Asetoaminofen ve lipopolisakkarit ilişkili böbrek hasarında Anadolu şurubu'nun histopatolojik parametreler üzerinde koruyucu etkisi olduğu gösterilmiştir.

**Anahtar kelimeler:** Akut böbrek hasarı, Anadolu şurubu, asetoaminofen, lipopolisakkkarit, histopatoloji, bitkisel tedavi

## INTRODUCTION

Acute kidney injury (AKI) is an acute disease that develops as a result of different etiologies and pathological mechanisms, with a high mortality rate (1). Although the pathogenesis of AKI has not been fully understood, renal inflammation, hypoxia, oxidative stress, mitochondrial dysfunction, and venous congestion play an important role (2,3). Since there is no effective treatment for acute kidney injury, new treatment approaches are needed (2).

The kidney contains many xenobiotic enzymes and has important roles in drug metabolism (4). Acetaminophen-N-acetyl-para-aminophenol (APAP) is an analgesic-antipyretic anti-inflammatory drug widely used in many countries around the world, and its overdose or frequent use causes hepatotoxicity and nephrotoxicity (5-7). Oxidative stress and reactive oxygen derivatives play an important role in APAP-associated hepatorenal damage in humans and animals (5,6). Although there are many publications on APAP-associated hepatotoxicity, there are few publications on nephrotoxicity (7). For this reason, it has been reported that natural compounds with antioxidant effects can be used as alternative treatment methods in APAP toxicity (5).

Sepsis is a systemic inflammatory response syndrome that occurs after microbial infections, and one of its major complications is acute kidney injury (1-3). Lipopolysaccharide (LPS), which is widely used in experimental sepsis-associated animal models, is a pathogenic endotoxin found in the outer membrane of gram-negative bacteria (1,2). LPS increases the production of proinflammatory cytokines such as tumor necrosis factor α (TNF-α), interleukin (IL-1β), and IL-6, as well as contributes to the formation of oxidative stress which has a key role in kidney injury (2,8).

Medicines obtained from plant extracts are widely used in the treatment of clinical diseases due to their natural antioxidant properties (4,9,10). Since drug toxicity causes serious cellular degeneration, it is thought that herbal combination products with fewer side effects and higher efficacy can be used as alternative treatment methods to protect the kidneys (9,11).

Anatolian syrup (AS) is a compound containing herbal oils (such as black cumin, ginger, cinnamon, grape seed, garlic, and rosemary). The beneficial effects of the vegetable oils and the primary compound in AS have been observed in many diseases such as hypertension, asthma, diabetes, inflammation, tumor, headache, diarrhea, skin diseases (1,5,10,12,13). There are many studies on the use of oils in AS in traditional treatment (14,15). Black cumin is a traditional medicine with antihypertensive, hypoglycemic, hypolipidemic, diuretic, antioxidant, and antibacterial properties, which is widely used in many diseases such as asthma, hypertension, diabetes mellitus, inflammation, tumors, bronchitis, headaches, fever, diarrhea, skin diseases, etc. (5,10). Few studies have demonstrated its protective effect against APAP-associated nephrotoxicity (6). Ginger has antioxidant properties along with hepatoprotective and nephroprotective effects (16). Grape seed, garlic, and rosemary have antioxidant, antimicrobial, anticancer, anti-inflammatory, and cardioprotective effects (4,13,17). It has been reported that pomegranate has antioxidant, antifungal, and anti-inflammatory effects (18). Oleuropein is the most dominant polyphenolic content of olive tree leaves, and it has antiviral, antioxidant, anti-inflammatory, anti-apoptotic, neuroprotective, and renoprotective effects (1,12,19).

The study aimed to demonstrate the protective effect of AS on histopathologically occurring

tissue damage in APAP and LPS-associated acute kidney injury. Histopathological variables like tubular atrophy, tubular dilatation, cytoplasmic vacuolization in tubular epithelial cells, tubular epithelial cell necrosis, interstitial inflammation, congestion, bleeding, glomerular damage, and brush border loss were used to measure the damage to the tissue.

## MATERIALS AND METHODS

### 1-Animals

In this study, 40 male Wistar albino rats (200-220 g, 2-4 months old) were used because they have a more toxic response to acetaminophen. Bolu Abant Izzet Baysal University, Experimental Animals Application and Research Center (DEHAM) provided animals, and feeding was performed with ad libitum water and pelleted feeds under 19±2°C and 50-70 degrees relative humidity using UNTES brand ventilation system. The laboratory methods and systems with which the study was going to be conducted were optimized for rats. Ethics committee approval was obtained from The Institutional Animal Care and Use Committee of Bolu Abant Izzet Baysal University (Bolu, Turkey) (Number: 2021/33). All procedures complied with the Guide for the Care and Use of Laboratory Animals (1996).

### 2-Preparation of Anatolian Syrup

AS is a compound produced by Muhammet Efe in Turkey, which contains essential oils of olive oil maceration and oil with oleuropein. The minimum and maximum concentrations of the oils in AS are as follows: Wormwood oil 1-3%, mint oil 0.5% -1.5%, black cumin oil 1-3%, pumpkin seed oil 1-3%, St. John's Wort oil 1-3%, African geranium oil %0.1-0.6%, grape seed oil 1-3%, oregano oil 1-3%, eucalyptus oil 0.5-1.5%, cinnamon oil 0.5-1.5%, garlic oil 1-3%, clove oil 0.5-1.5%, bitter almond oil 1-3%, ginger oil 1-3%, potency pomegranate oil 3-9%, pomegranate oil 0.5-1.5%, walnut oil 1%-3, orange peel oil 1-3%, horseradish oil 1-3%, rosemary 0.5-1.5%. The total of olive oil maceration essential oils is 18.1-54.6%. The ratio of olive oil with oleuropein is 45.9-81.9%.

### 3-Creation of groups

In the study, there were 5 groups in total: Sham (control) group (n=8): (0.3 ml/day distilled water, orally for three days), acetaminophen (APAP) group (n=8): (1 g/kg paracetamol single dose intraperitoneal) (7), lipopolysaccharide (LPS) group (n=8): (5 mg/kg Lipopolysaccharide single dose intraperitoneal) (20), acetaminophen+Anatolian syrup (APAP + AS) group (n=8): (oral administration of anatolian syrup 1g/kg for 15 days, then 1 g/kg APAP single dose intraperitoneal), lipopolysaccharide+Anatolian syrup (LPS+AS) group (n=8): (after 15 days of oral administration of Anatolian syrup 1 g/kg, 5 mg/kg LPS single dose, intraperitoneal).

### 4-Histopathological examination

For histopathological examination, rat kidney tissues were fixated in 10% formalin. The samples taken from the tissues were taken into tissue follow-up and embedded in paraffin blocks after the follow-up. Sections of 3 µm thickness were taken from the prepared paraffin blocks and stained with hematoxylin-eosin stain. The pathologist examined sections under the LEICA brand DM 2000 LED light microscope. Microscopically in kidney tissue, tubular atrophy, tubular dilation, cytoplasmic vacuolization in tubular epithelial cells, tubular epithelial cell necrosis (luminal necrotic debris), interstitial inflammation, vascular congestion, hemorrhage and glomerular damage, and loss of brush border were scored from 0 to 5 semiquantitatively (0-5%=score 0, 6-20%=score 1, 21-40%=score 2, 41-60%=score 3, 61-80%=score 4, 81-100%=score 5) (5). Hematoxylin-eosin-stained sections were imaged at different magnifications with the INFINITY 3 ANALYZE Release 6.5 imaging system.

### Statistical analyses

Descriptive statistics were obtained for the variables. Before statistical analyses, distributional properties of the continuous variables were

evaluated using the Shapiro-Wilk test. Groups were compared using the Kruskal–Wallis test, and Mann–the Whitney U test was employed as a post-hoc test for non-normally distributed variables. Bonferroni correction was applied due to multiple comparisons. A p-value of <0.05 was considered to be statistically significant. All statistical analyses were carried out using the R software (version 4.1.0).

## RESULTS

As a result of the Shapiro-Wilk test, it was understood that the variables demonstrating continuous variation did not show normal distribution. As a result, the descriptive statistics were given as median (min.-max.) Kruskal Wallis test in multiple group comparisons and Mann–Whitney U test as a post-hoc test were used.

The analysis of the histopathological data of the groups can be found in Table 1, and the distribution of the groups is shown in Graphic 1. There was a statistically significant difference between the groups in tubular atrophy (p<0.001). There was a statistically significant increase in the APAP, LPS, and LPS+APAP groups compared to the sham group. A statistically significant decrease was observed in the APAP+AS group compared to the APAP group. There was no statistically significant difference between the sham and APAP+AS groups. A statistically significant decrease was observed in the LPS+AS group compared to the LPS group. LPS+AS group was statistically significantly higher than the sham group.

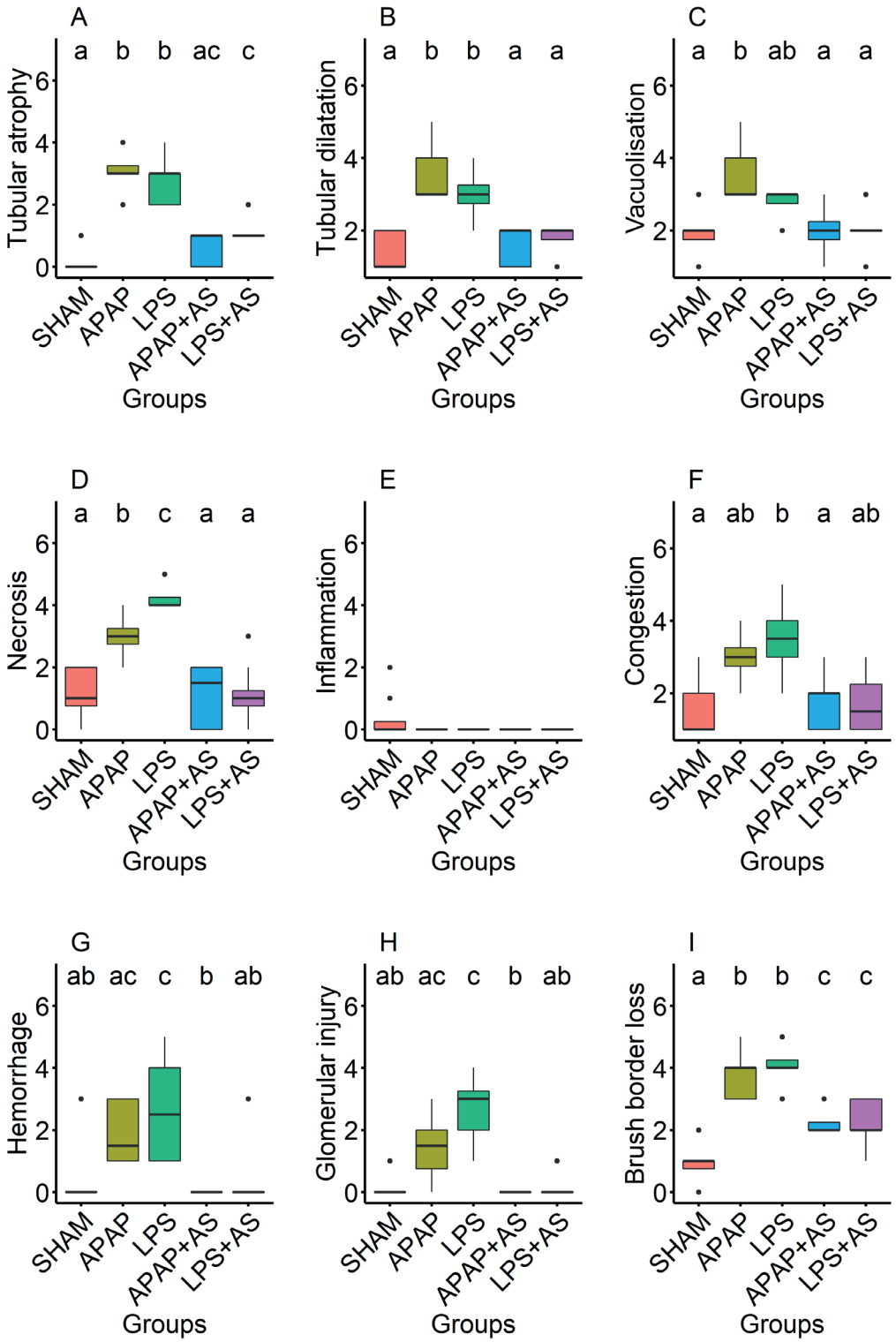
There was a statistically significant difference between the groups in tubular dilatation (p<0.001). There was a statistically significant increase in the APAP and LPS groups compared to the sham group. There was a statistically significant decrease in the APAP+AS group compared to the APAP group. There was no statistically significant difference between the APAP+AS, and the Sham group. There was a statistically significant decrease in the LPS+AS group compared to the LPS group. There was no statistically significant difference between the LPS+AS and the Sham group.

There was a statistically significant difference between the groups in cytoplasmic vacuolization (p<0.001). A statistically significant increase was observed in the APAP group compared to the sham group. There was a statistically significant decrease in the APAP+AS group compared to the APAP group. There was no statistically significant difference between the APAP+AS and sham groups. A slight increase was observed in the LPS group compared to the sham group, which was not statistically significant. A statistically insignificant decrease was observed in the LPS+AS group compared to the LPS group. There was no statistically significant difference between the LPS+AS and sham groups.

**Table 1. Descriptive statistics* of histopathological variables.**

| Variables | Sham (n=8) | APAP (n=8) | LPS (n=8) | APAP+AS (n=8) | LPS+AS (n=8) | p |
|---|---|---|---|---|---|---|
| Tubular atrophy | 0.0(0.0-1.0)$^{a}$ | 3.0(2.0-4.0)$^{b}$ | 3.0(2.0-4.0)$^{b}$ | 1.0(0.0-1.0)$^{ac}$ | 1.0(1.0-2.0)$^{c}$ | 0.000 |
| Tubular dilation | 1.0(1.0-2.0)$^{a}$ | 3.0(3.0-5.0) $^{b}$ | 3.0(2.0-4.0)$^{b}$ | 2.0(1.0-2.0)$^{a}$ | 2.0(1.0-2.0)$^{a}$ | 0.000 |
| Cytoplasmic vacuolization | 2.0(1.0-3.0)$^{a}$ | 3.0(3.0-5.0) $^{b}$ | 3.0(2.0-3.0)$^{ab}$ | 2.0(1.0-3.0)$^{a}$ | 2.0(1.0-3.0)$^{a}$ | 0.000 |
| Necrosis | 1.0(0.0-2.0)$^{a}$ | 3.0(2.0-4.0) $^{b}$ | 4.0(4.0-5.0)$^{c}$ | 1.5(0.0-2.0)$^{a}$ | 1.750(0.0-3.0)$^{a}$ | 0.000 |
| Inflammation | 0.0(0.0-2.0) | 0.0(0.0-0.0) | 0.0(0.0-0.0) | 0.0(0.0-0.0) | 0.0(0.0-0.0) | 0.084 |
| Congestion | 1.0(1.0-3.0)$^{a}$ | 3.0(2.0-4.0)$^{ab}$ | 3.5(2.0-5.0)$^{b}$ | 2.0(1.0-3.0)$^{a}$ | 1.5(1.0-3.0)$^{ab}$ | 0.000 |
| Hemorrhage | 0.0(0.0-3.0)$^{ab}$ | 1.5(1.0-3.0)$^{ac}$ | 2.5(1.0-5.0)$^{c}$ | 0.0(0.0-0.0)$^{b}$ | 0.0(0.0-3.0)$^{ab}$ | 0.000 |
| Glomerular damage | 0.0(0.0-1.0)$^{ab}$ | 1.5(0.0-3.0)$^{ac}$ | 3.0(1.0-4.0)$^{c}$ | 0.0(0.0-0.0)$^{b}$ | 0.0(0.0-1.0)$^{ab}$ | 0.000 |
| Brushy edge loss | 1.0(0.0-2.0)$^{a}$ | 4.0(3.0-5.0)$^{b}$ | 4.0(3.0-5.0)$^{b}$ | 2.0(2.0-3.0)$^{c}$ | 2.0(1.0-3.0)$^{c}$ | 0.000 |

**: Values are median (min-max). P-value was obtained as a result of Kruskal Wallis analysis. Mann Whitney U test was used to determine the different group(s). Bonferroni correction was performed for multiple comparisons. There was a statistically significant difference between the group(s) that did not have the same letter for each variable (P<0.05).*
*APAP: Acetaminophen, LPS: Lipopolysaccharide, AS: Anatolian syrup*




**Graph 1: Distributional properties of each variable are presented as boxplot graphic.**

*A) Tubular atrophy, B) Tubular dilatation, C) Vacuolization, D) Necrosis, E) Inflammation, F) Congestion, G) Hemorrhage, H) Glomerular injury, I) Brush border loss. There was a statistically significant difference between the group(s) that did not have the same letter for each variables (P<0.05)*

There was a statistically significant difference between the groups in necrosis ($p<0.001$). A statistically significant increase was observed in the LPS and APAP groups compared to the sham group. There was a statistically significant decrease in the LPS+AS group compared to the LPS group. There was a statistically significant decrease in the APAP+AS group compared to the APAP group.

A statistically significant difference was observed between the groups in congestion ($p<0.001$). A statistically significant increase was observed in the LPS group compared to the sham group. Although an increase was observed in the APAP group compared to the sham group, it was not statistically significant. Although a decrease was observed in the LPS+AS group compared to the LPS group, it was not statistically significant. Although a decrease was observed in the APAP+AS group compared to the APAP group, was not statistically significant. There was no significant difference between the sham group and the LPS+AS and APAP+AS groups.

A statistically significant increase was observed in hemorrhage in the LPS, and APAP groups compared to the sham group ($p<0.001$). There was a statistically significant decrease in the LPS+AS group compared to the LPS group. There was a statistically significant decrease in the APAP+AS group compared to the APAP group. There was no statistically significant difference between the Sham group and the LPS+AS and APAP+AS groups.

There was a significant difference between the groups in glomerular damage ($p<0.001$). A statistically significant increase was observed in the LPS group compared to the sham group. There was a statistically significant decrease in the LPS+AS group compared to the LPS group. There was no statistically significant difference between the LPS+AS and the sham groups. Although the increase was observed in the APAP group compared to the sham group, it was not statistically significant. There was a statistically significant decrease in the APAP+AS group compared to the APAP group. There was no statistically significant difference between the APAP+AS and the sham groups.

Loss of brush border demonstrated a statistically significant difference between the groups ($p<0.001$). Loss of brush border in the LPS and APAP groups was statistically significantly higher than in the sham group (median (min-max, respectively). There was a statistically significant increase in the LPS group compared to the sham group. There was a statistically significant decrease in the LPS+AS group compared to the LPS group. However, the decrease in the LPS+AS group was not as much as in the sham group. A statistically significant decrease was observed in the APAP+AS group compared to the APAP group. However, the decrease in the APAP+AS group was not as much as in the sham group. Inflammation was observed only in the control group, and no inflammation was observed in other groups. Microscopic images of the groups are given in Figure 1 and Figure 2.

## DISCUSSION

Acute kidney injury is characterized by impaired renal function, fluid volume and electrolytes changes, and an increase in serum creatinine levels (12). Since drug toxicity causes serious cellular degeneration, it is thought that herbal products with fewer side effects and higher efficacy can be used as alternative treatment methods in the prevention and treatment of acute kidney injury (11,12).

Anatolian syrup is a herbal compound containing many oils, and many studies have been conducted on the efficacy of vegetable oils in its content (9,21). Parkar et al.[21] showed that orange reduces renal damage and fibrosis in diabetic nephropathy. Barakat et al.[22] demonstrated that pumpkin seeds have hypolipidemic and immune system regulatory effects. Extracts or oils obtained from eucalyptus leaves have antifungal, antibacterial, anti-inflammatory and antioxidant effects (14).

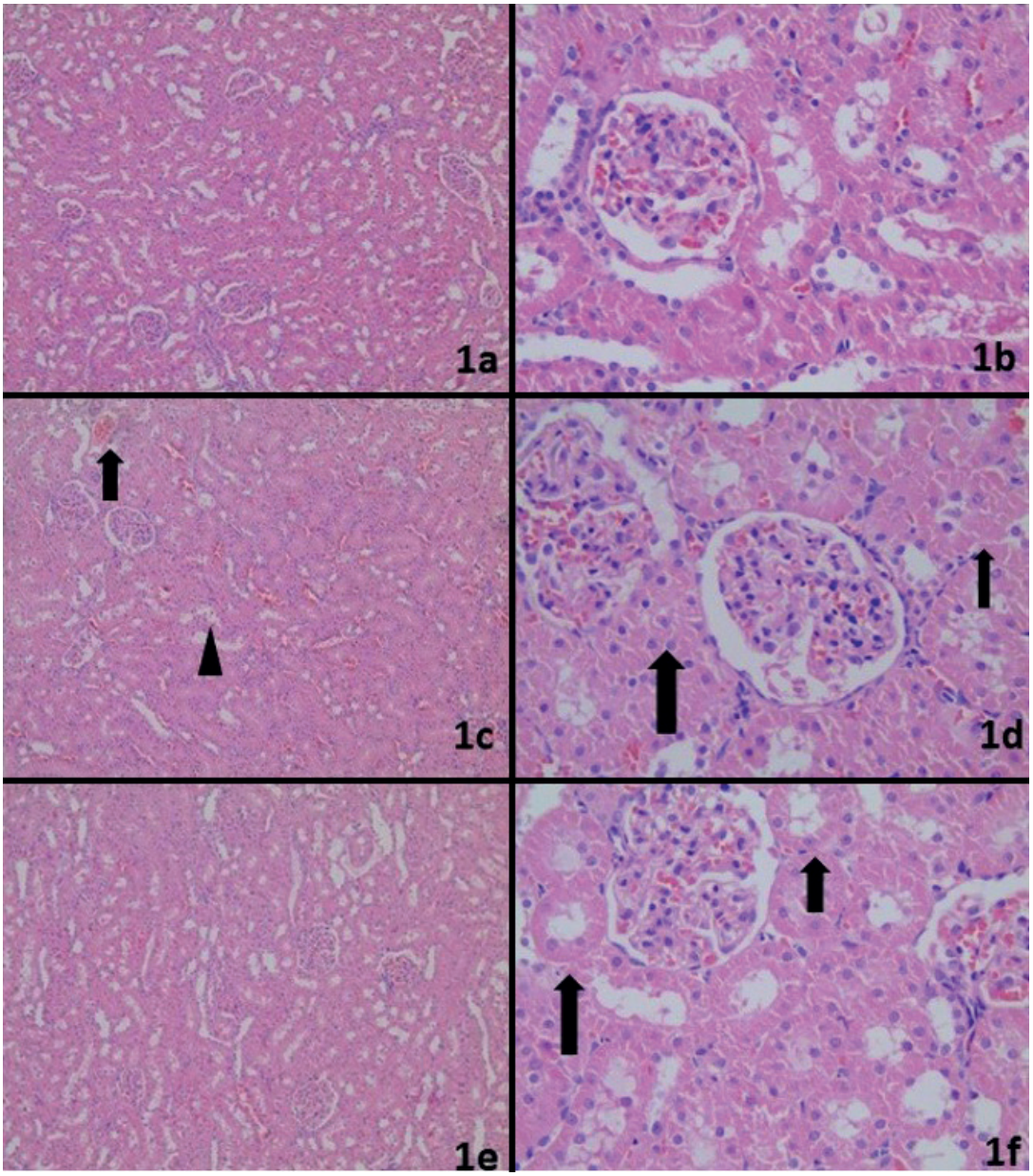



**Figure 1. 1a: Sham x100, 1b: Sham x400, 1c: APAP x100 vascular congestion (arrow), tubular dilatation (arrowhead), 1d: APAP x400 tubular epithelial cell necrosis (arrow), 1e: APAP+AS x100, 1f: APAP+AS x400 no significant necrosis in tubular epithelial cells (arrow).**

It has been reported that APAP and LPS can have nephrotoxic effects (2,5,6). LPS plays a role in the pathogenesis of sepsis-associated acute kidney injury by increasing inflammation and oxidative stress (2). Acute APAP overdose increases lipid peroxidation concerning increased radical oxygen species (ROS) and weakens antioxidant defense mechanisms and causes histopathological damage on the renal structure (14). Inflammatory cytokines play an important role in APAP toxicity, and elevated levels of serum TNF-alpha, and IL-1-β have been reported (6). Canayakin et al.[5] demonstrated in their studies that black cumin has an important role in the nephrotoxic damage of APAP. It has been shown that black cumin reduces necrosis, congestion, and tubular dilatation in kidney tissue (5). In our study, our histopathological results were consistent with this study.

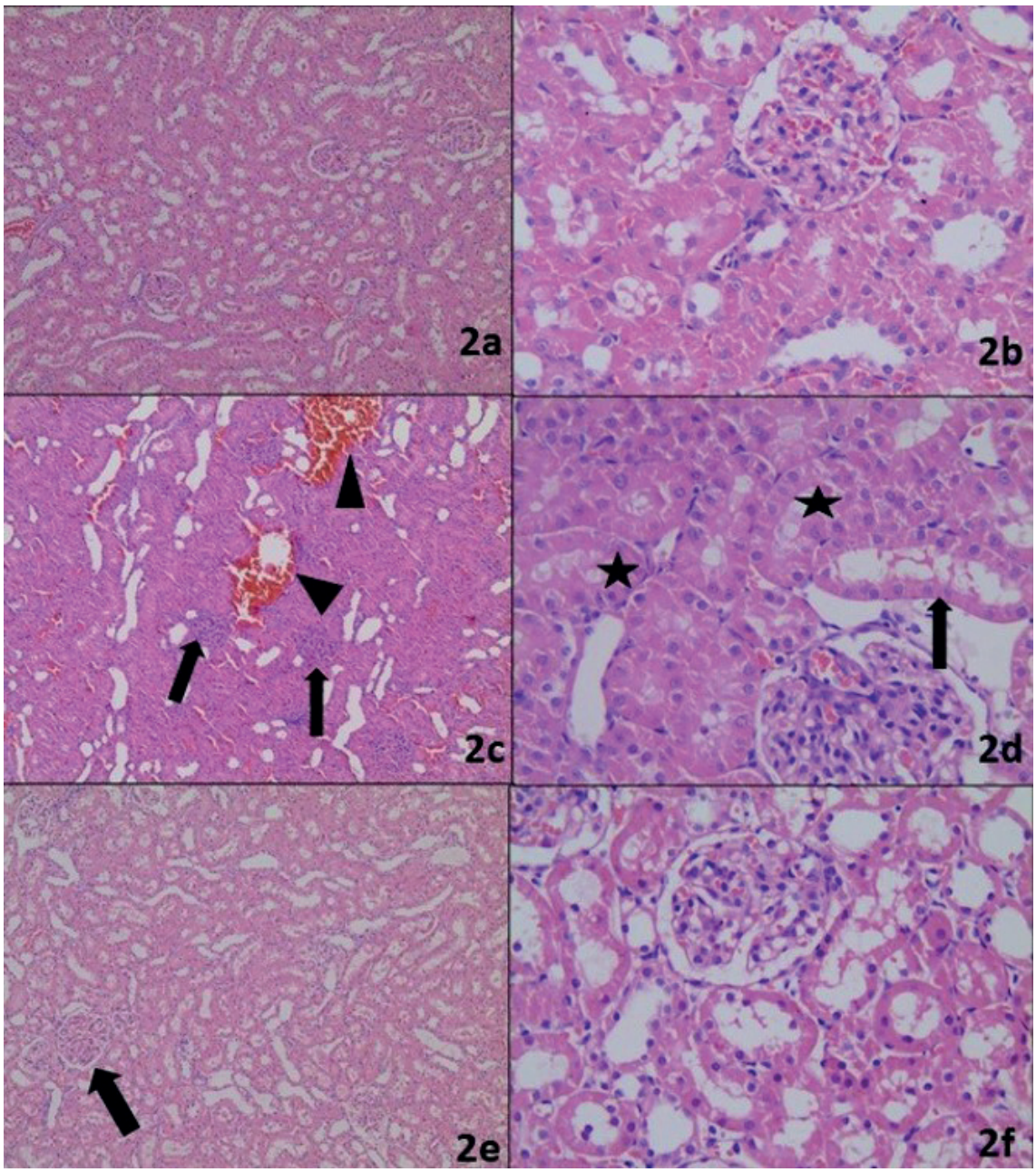



**Figure 2. 2a: Sham x100 2b: Sham x400, 2c: LPS x100 glomerular damage (arrow) and apparent vascular congestion (arrowhead), 2d: LPSX400 tubular dilatation (arrow), necrosis and luminal necrotic debris in tubular epithelial cell (asterisk), 2e: LPS+AS x100, 2f: LPS x400.**

In the study by Albarakati[17], garlic extract reduced apoptotic and necrotic changes in kidney tissue in ethephon-associated kidney injury. Rosemary oil (4), mint (23), and wormwood oil (15) have been shown to reduce histopathological damage in the kidney. The study by Mousavi[10] showed that pretreatment with black cumin histopathologically improved hemorrhage in renal ischemia-reperfusion injury, tubular necrosis, cytoplasmic vacuolization, interstitial edema, hyperemia, and glomerular changes. Hassan et al.[13] stated in their study on rats that grape seed has a hepatonephroprotective effect by showing that it reduced tissue damage and inflammation histopathologically.

Abdeen et al.[7] investigated the efficacy of cinnamon in APAP-associated kidney injury, and it was shown that the loss of brush border, hydropic degeneration, tubular vacuolization and lymphocytic inflammation in the kidney were reduced in the APAP group. It has been stated that cinnamon has this effect by protecting the lipid peroxidation caused by high-dose APAP from DNA damage and apoptosis (7). Bakir et al.[18] showed that in cisplatin-associated nephrotoxicity, pomegranate is protective against kidney damage by providing improvements in morphological variables such as swelling in tubular epithelial cells, loss of brush border, and loss of tubular epithelial cells. Dhibi et al.[14] demonstrated that pretreatment with eucalyptus improves changes in the proximal tubule, glomerular atrophy, and dilatation in acetaminophen associated kidney injury. Yin et al.[12] reported that oleuropein inhibits tubular dilatation, vacuolization, glomerular hypertrophy in kidney tissue, and debris and inflammation in the renal tubule lumen in glycerol associated with kidney injury. It has been stated that oleuropein can be used in the treatment of acute kidney injury (12). Cui et al.[1] applied oleuropein to lipopolysaccharide-associated kidney injury in mice and histologically improved tubular epithelial cell swelling and deformation. Alsharif et al.[8] showed that oleuropein reduces tubular degeneration, glomerular congestion, and inflammatory infiltration in LPS damage. Koc et al.[11] showed that oleuropein improves tubular degeneration, tubular vacuolization, tubular dilatation, inflammation, and congestion in rat kidney tissue, which are caused by indomethacin, a nonsteroidal anti-inflammatory drug. It showed an antiapoptotic effect by suppressing renal caspase-3 expression (11). Oleuropein has been shown to reduce both tubular and glomerular damage in cisplatin-associated kidney injury (19). Lee et al.[24] showed that in colistin-associated kidney damage, garlic reduces renal tubule damage through apoptosis and has antioxidant and anti-inflammatory effects.

In our study, increased tubular atrophy, tubular dilatation, cytoplasmic vacuolization, necrosis, vascular congestion, hemorrhage, glomerular damage, and loss of brush border were seen in the APAP and LPS groups, resulting in histologic damage. In the APAP+AS and LPS+AS groups, these variables were improved in line with the literature (1,11,12,18). Since inflammation was seen only in the sham group, a comparison could not be made. The nephroprotective effect of AS was observed.

## CONCLUSIONS

It was shown that AS has a protective effect on histopathological variables in APAP and LPS-associated kidney damage. We believe that by investigating the antioxidant and anti-inflammatory effects of AS on the kidney with more comprehensive clinical studies involving the use of different doses of AS, AS can be a potential therapeutic agent that can be used in the treatment of acute kidney injury.

**Limitations of the study:** The limitations of the study were the inability to examine kidney functions, antioxidant, and anti-inflammatory effects.

**Ethics Committee Approval:** The study was approved by Bolu Abant Izzet Baysal University Ethic Committee for Clinical Research (2021/33).

**Conflict of Interest:** No conflict of interest was declared by the authors.

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## REFERENCES

1. Cui Y, Gao H, Han S, Yuan R, He J, Zhuo Y. Oleuropein Attenuates Lipopolysaccharide-Induced Acute Kidney Injury In Vitro and In Vivo by Regulating Toll-Like Receptor 4 Dimerization. Front Pharmacol 2021;12: 617314. https://doi.org/10.3389/fphar.2021.617314

2. Feng X, Wang C, Fan H. Dexmedetomidine ameliorates lipopolysaccharide - induced acute kidney injury in rats by inhibiting inflammation and oxidative stress via the GSK - 3 β / Nrf2 signaling pathway. J Cell Physiol 2019;234(10): 18994-19009. https://doi.org/10.1002/jcp.28539
3. Ding Y, Zheng Y, Huang J, Peng W, Chen X, Kang X. UCP2 ameliorates mitochondrial dysfunction, inflammation, and oxidative stress in lipopolysaccharide-induced acute kidney injury. Int Immunopharmacol 2019;71: 336-49. https://doi.org/10.1016/j.intimp.2019.03.043
4. Hassanen NHM, Fahmi A, Shams-eldin E. Protective effect of rosemary (Rosmarinus officinalis) against diethylnitrosamine-induced renal injury in rats. Biomarkers 2020;25(3): 281-9. https://doi.org/10.1080/1354750X.2020.1737734
5. Canayakin D, Bayir Y, Baygutalp NK, Karaoglan ES, Atmaca HK, Ozgeris FBK et al. Paracetamol-induced nephrotoxicity and oxidative stress in rats: the protective role of Nigella sativa. Pharm Biol 2016;54(10): 2082-91. https://doi.org/10.3109/13880209.2016.1145701
6. Wang X, Wu Q, Liu A, Anadón A, Rodríguez J, Yuan Z, et al. Paracetamol: overdose-induced oxidative stress toxicity, metabolism, and protective effects of various compounds in vivo and in vitro. Drug Metab Rev 2017;49(4): 395-437. https://doi.org/10.1080/03602532.2017.1354014
7. Abdeen A, Abdelkader A, Abdo M, Wareth G, Aboubakr M, Aleya L et al. Protective effect of cinnamon against acetaminophen-mediated cellular damage and apoptosis in renal tissue. Environ Sci and Pollut Res 2019;26(1): 240-9. https://doi.org/10.1007/s11356-018-3553-2
8. Alsharif K, Almalki A, Al-Amer O, Mufti A, Theyab A, Lokman M et al. Oleuropein protects against lipopolysaccharide-induced sepsis and alleviates inflammatory responses in mice. IUBMB Life 2020;72(10): 2121-32. https://doi.org/10.1002/iub.2347
9. Rajagopalan P, Ashraf AE, Essam MA. Chamomile and Oregano extracts synergistically exhibits anti hyperglycemic, antihyperlipidaemic and renal protective effects in Alloxan induced Diabetic rats. Can J Physiol and Pharmacol 2016;95(1): 84-92. https://doi.org/10.1139/cjpp-2016-0189
10. Mousavi G. Study on the effect of black cumin (Nigella sativa Linn.) on experimental renal ischemia- reperfusion injury in rats. Acta Cir Bras 2015;30(8): 542-50. https://doi.org/10.1590/S0102-865020150080000005
11. Koc K, Cerig S, Ozek NS, Aysin F, Yildirim S, Ckmak O et al. The efficacy of oleuropein against non-steroidal anti- inflammatory drug induced toxicity in rat kidney. Environ Toxicol 2019;34(1): 67-72. https://doi.org/10.1002/tox.22658
12. Yin M, Jiang N, Guo L, Ni A, Al-Brakati AY, Othman MS et al. Oleuropein suppresses oxidative, inflammatory, and apoptotic responses following glycerol-induced acute kidney injury in rats. Life Sci 2019;232: 116634. https://doi.org/10.1016/j.lfs.2019.116634
13. Hassan HA, Al-rawi MM. Grape seeds proanthocyanidin extract as a hepatic-reno-protective agent against gibberellic acid induced oxidative stress and cellular alterations. Cytotechnology 2013;65: 567-76. https://doi.org/10.1007/s10616-012-9506-6
14. Dhibi S, Mbarki S, Elfeki A, Hfaiedh N. Eucalyptus globulus extract protects upon acetaminophen-induced kidney damages in male rat. Bosn J Basic Med Sci 2014;14(2): 99-104. https://doi.org/10.17305/bjbms.2014.2272
15. Saoudi M, Badraoui R, Rahmouni F, Jamoussi K, El Feki A. Antioxidant and Protective Effects of Artemisia campestris Essential Oil Against Chlorpyrifos-Induced Kidney and Liver Injuries in Rats. Front Physiol 2021;12(194): 1-10. https://doi.org/10.3389/fphys.2021.618582
16. Abdel-Gabbar M, Ahmed RR, Kandeil MA, Mohamed AE deen H, Ali SM. Administration of ginger and / or thyme has ameliorative effects on liver and kidney functions of V-line rabbits: Histological and biochemical studies. J Anim Physiol Anim Nutr 2019;(April):1758-67. https://doi.org/10.1111/jpn.13166
17. Albrakati A. Aged garlic extract rescues ethephon-induced kidney damage by modulating oxidative stress, apoptosis, inflammation, and histopathological changes in rats. Environ Sci Pollut Res 2021;28(6): 6818-29. https://doi.org/10.1007/s11356-020-10997-5
18. Bakır S, Yazgan UC, Ibiloglu I, Elbey B, Kızıl M. The protective effect of pomegranate extract against cisplatin toxicity in rat liver and kidney tissue. Arch of Physiol Biochem 2015;121(4): 152-6. https://doi.org/10.3109/13813455.2015.1068336
19. Geyikoglu F, Emir M, Colak S, Koc K, Turkez H, Bakir M et al. Effect of oleuropein against chemotherapy drug-induced histological changes, oxidative stress, and DNA damages in rat kidney injury. J Food Drug Anal 2017;25(2): 447-59. https://doi.org/10.1016/j.jfda.2016.07.002
20. Rahimi VB, Rakhshandeh H, Raucci F, Buono B, Shirazinia R, Kermani AS et al. Anti-Inflammatory and Anti-Oxidant Activity of Portulaca oleracea Extract on LPS-Induced Rat Lung Injury. Molecules 2019;24(1): 139. https://doi.org/10.3390/molecules24010139
21. Parkar N, Addepalli V. Amelioration of diabetic nephropathy by orange peel extract in rats. Nat Prod Res 2014;28(23): 2178-81. https://doi.org/10.1080/14786419.2014.925894

22. Barakat LAA, Mahmoud RH. The antiatherogenic, renal protective and immunomodulatory effects of purslane, pumpkin and flax seeds on hypercholesterolemic rats. N Am J Med Sci 2011;3(9): 351-7. https://doi.org/10.4297/najms.2011.3411
23. Bellassoued K, Hsouna AB, Athmouni K, Pelt JV, Ayadi FM, Rebai T et al. Protective effects of Mentha piperita L. leaf essential oil against CCl 4 induced hepatic oxidative damage and renal failure in rats. Lipids Health Dis 2018;17(9):1-14. https://doi.org/10.1186/s12944-017-0645-9
24. Lee TW, Bae E, Kim JH, Jang HN, Cho HS, Chang SH et al. The aqueous extract of aged black garlic ameliorates colistin-induced acute kidney injury in rats. Ren Fail 2019;41(1):24-33. https://doi.org/10.1080/0886022X.2018.1561375