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Original Article

Nephroprotective effects of ethanol leaf extract of *Stachys pilifera* Benth in alcohol-induced nephrotoxicity in male rats

Ali Taheri Mirghaed[1], Narsis Khoshkharam[1], Farzaneh Karimi[2], Soroor Abdzadeh[1], Marzieh Razazan[3], Zahra Rahami[3,*], and Amir Hossein Doustimotlagh[4,*]

[1]*Student Research Committee, Yasuj University of Medical Sciences, Yasuj, I.R. Iran.*
[2]*Behbehan Faculty of Medical Sciences, Behbehan, I.R. Iran.*
[3]*Department of Internal Medicine, Faculty of Medicine, Yasuj University of Medical Sciences, Yasuj, I.R. Iran.*
[4]*Department of Clinical Biochemistry, Faculty of Medicine, Yasuj University of Medical Sciences, Yasuj, I.R. Iran.*

Abstract

Background and purpose: Excessive and long-term consumption of alcohol causes health problems and significant changes in the physiological and biochemical functions of the body organs, including the kidneys. The present study aimed to clarify and investigate the antioxidant and anti-inflammatory impacts of *Stachys pilifera* Benth (SPB) hydroalcoholic extract on ethanol (EtOH)-induced nephrotoxicity.

Experimental approach: Twenty-four rats were randomly divided into 4 groups. The control group was administered distilled water as a vehicle for 35 days. The EtOH group received ethanol (40% v/v, 7 mL/kg) orally for 35 days. The SPB extract + EtOH group was pretreated with 500 mg/kg of SPB orally and, after 1 h, received ethanol (40% v/v, 7 mL/kg) orally for 35 days; the SPB extract group received only 500 mg/kg of SPB for 35 days. After 35 days, the rats were sacrificed, and the blood sample was taken. Then, levels of biochemical markers, oxidative stress indices, antioxidant enzyme activity, and pro-inflammatory cytokines were evaluated.

Findings/Results: In the EtOH group, the levels of BUN, Cr, NO metabolite, and MDA were significantly higher compared to the control group. In addition, a significant elevation in the levels of pro-inflammatory cytokines (TNF-α and IL-6) was observed compared to the control group. Pretreatment with SPB hydroalcoholic extract led to significant diminishment in BUN, Cr, MDA, NO metabolite, and TNF-α and IL-6.

Conclusion and implications: The hydroalcoholic extract of SPB led to improvement of kidney function, reduction of oxidative stress, and improvement of inflammatory conditions in nephrotoxicity caused by ethanol in male rats.

Keywords: Anti-inflammatory; Antioxidant; Ethanol; Nephrotoxicity; Rat; *Stachys pilifera* Benth.

INTRODUCTION

Depending on the amount and duration of consumption, alcohol can cause diseases such as liver diseases, diabetes, high blood pressure, cardiovascular diseases, stroke, different types of cancers, pneumonia, and mental health and behavioral disorders (1-5). Alcohol abuse leads to impressive changes in the physiological and biochemical functions of the body organs, such as the liver, pancreas, and kidneys (6,7). Ethanol abuse leads to structural and functional variations in the kidneys (8). Alcohol disrupts the ability of the kidneys to regulate fluid-electrolyte balance and leads to drastic changes in the body's acid-base balance (9). Furthermore, an increase in the concentration of blood urea nitrogen (BUN) and creatinine (Cr) has been reported in excessive alcohol consumption (10).

*Correspondence authors:
A. H. Doustimotlagh: Tel: +98-7433346070, Fax: +98-7433346071
Email: amirhosseindoustimotlagh@gmail.com
Z. Rahami: Tel: +98-7433346070, Fax: +98-7433346071
Email: z.rahami.65@gmail.com

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The findings demonstrated that the toxicity arising from alcohol consumption is due to some mechanisms, such as inflammation, oxidative stress, apoptosis, or other signaling pathways (11,12). Oxidative stress and toxicity caused by reactive oxygen species (ROS) are considered one of the important pathways of kidney damage in excessive alcohol consumption (13).

Ethanol is mainly metabolized in the liver through different pathways, but the metabolic pathway cytochrome P450 2E1 leads to oxidative stress and tissue injury by forming ROS. Cytochrome P450 2E1 is expressed in the kidneys and catabolizes ethanol and generates ROS (14). In addition, high and long-term alcohol consumption leads to a significant increase in oxidative stress with hyperacetylation of mitochondrial proteins in the kidneys (11). Due to the great quantity of polyunsaturated fatty acids (PUFAs) in the structure of renal lipids, the kidney is a very vulnerable organ against oxidative stress (15). Cells can tolerate and deal with mild oxidative stress due to their antioxidant system. But in more severe cases, ROS vanquish antioxidant systems and cause harmful changes in the structure and function of vital biomolecules such as DNA, proteins, and lipids, leading to cellular damage (16). Oxidative stress causes a wide range of kidney failure, including rhabdomyolysis, obstructive nephropathy, acute and chronic kidney failure, and glomerular damage (13). Therefore, controlling and dealing with oxidative stress is one of the important solutions to preventing kidney failure caused by alcohol consumption (17).

Phytocompounds can diminish the risk of cell injury caused by ROS. Although phytocompounds are present in very small quantities, it has been reported that they can affect cellular functions and play a crucial role in diminishing inflammation and oxidative stress. The utilization of herbal treatment against diverse chronic ailments induced by alcohol abuse has been a routine clinical cure for a long time in some countries (18). *Stachys pilifera* Benth (SPB) is an aboriginal plant species in Iran that is used in folk remedies for the cure of various diseases, including rheumatoid arthritis and asthma. Also, this plant is used as an expectorant, pain reliever, and anti-infection (especially for women's infections). Some investigations revealed that SPB has anti-inflammatory, antioxidant, antibacterial, immunoregulatory, nephroprotective, and hepatoprotective properties (19,20). Despite extensive studies on nephrotoxicity in various models such as ischemia-reperfusion (IR) injury, acetaminophen, cisplatin, *etc*., the mechanism of kidney damage in the models differs from alcohol-induced nephrotoxicity, as follows: ethanol nephrotoxicity is caused by acetaldehyde metabolism, oxidative stress, and mitochondrial dysfunction (11). In contrast, cisplatin toxicity is more related to DNA damage and inflammation (21), acetaminophen damage is due to reactive metabolites N-acetyl-p-benzoquinone imine (NAPQI) (22), and IR injury is caused by ischemia followed by reperfusion *via* inflammation and oxidative stress (23). Ethanol affects renal function through ROS production and tubular cell apoptosis (14). Cisplatin and acetaminophen primarily cause tubular necrosis, whereas IR injury involves a complex process of hypoxia and oxidative damage with a strong inflammatory component (24-26) Oxidative stress and inflammation are involved in the forms of nephrotoxicity, but ethanol-induced nephrotoxicity is distinct due to its reliance on acetaldehyde and alcohol metabolism, whereas other nephrotoxins, such as cisplatin and acetaminophen, involve distinct metabolic pathways for their metabolic damage. Each type of nephrotoxicity also differs in terms of treatment approaches; ethanol-induced nephrotoxicity often requires interventions to manage alcohol toxicity and support renal function, while cisplatin and acetaminophen-induced toxicity may involve specific antidotes or therapies that target oxidative stress and inflammation. Therefore, in the present study, the nephroprotective effects of SPB. The hydroalcoholic extract in the ethanol-induced nephrotoxicity was investigated.

MATERIALS AND METHODS

Plant extraction

Aerial parts of SPB, such as leaves and stems, were procured in Spring 2023 from the

countryside of Yasuj, Iran. A botanist approved the plant (Herbarium No. 1897). The samples were cleaned and stored away from sunlight for several days until they were dry. Then, they were powdered and ready for extraction. To prepare the hydroalcoholic extract of SPB, 100 g of the powder was drenched in 1000 mL of ethanol (70% v/v) and placed at a temperature of 37 °C for 48 h. Then, the hydroalcoholic extract of SPB was filtered using Filter Paper No. 1, and the plant deposit was vaporized under condensed pressure by using a rotary evaporator at 60 °C. In the final step, the extract was dried and stored at -20 °C (27).

Animals

Twenty-four male adult Sprague-Dawley rats (180 ± 20 g) were provided from the Animal Colony of Shiraz University of Medical Sciences and maintained at 25 ± 2 °C with a controlled cycle of 12 h light/dark and free access to diet and water. The experiment was conducted in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals and approved by the Ethics Committee of Yasuj University of Medical Sciences (ethical code: IR.YUMS.AEC.1402.013).

Study design

Animals were randomly divided into 4 groups (n = 6). The control group was administered distilled water as a vehicle for 35 days. Ethanol (absolute Ethanol, 99.9%, Merck, Germany) group (EtOH) received ethanol (40% v/v, 7 mL/kg) orally for 35 days (28). SPB + EtOH group was pretreated with 500 mg/kg of SPB orally and, after 1 h, received ethanol (40% v/v, 7 mL/kg) orally for 35 days. The SPB group received only 500 mg/kg of SPB for 35 days (29). After 35 days, rats were intraperitoneally anesthetized with ketamine (60 mg/kg, Alfasan, Netherlands) and xylazine (5 mg/kg, Alfasan, Netherlands) and then sacrificed following ethical protocols based on approved guidelines for working with laboratory animals (30). Blood samples were collected in heparinized tubes for measurement of biochemical, oxidative stress, and inflammatory parameters. Kidneys were removed; one of the kidneys was separated into 2 pieces to prepare a homogenized tissue as well as for histopathological evaluation, and the other kidney was used to evaluate gene expression.

Biochemical analysis

For assessment of biochemical markers, blood heparinized tubes were centrifuged for 10 min at 3000 rpm, and creatinine (Cr) and blood urea nitrogen (BUN) were evaluated by standard diagnostic kits (Delta Co, Iran), and read by a biochemical autoanalyzer (BT-1500-A-A, Rome, Italy).

Measurement of oxidative stress markers

Lipid peroxidation assay

Based on Ohkawa's study, malondialdehyde (MDA) content was measured in kidney homogenate tissues using thiobarbituric acid (CAS Number: 504-17-6, Sigma, USA) (31). In this method, a total of 0.5 mL of tissue homogenate was mixed separately with 2 mL of a solution containing thiobarbituric acid (15% w/v), trichloroacetic acid (0.375% w/v, Sigma, USA), and 0.25 N HCl (Sigma, USA). The content was heated in a water bath at 100 °C for 15 min, then cooled for 10 min in cold water. After centrifuging at 2000 *g* for 15 min, the absorbance was read at 535 nm and expressed as µmol/g tissue (32).

Measurement of total thiol

Kidney homogenate total thiol (T-SH) was determined by the colorimetric method based on the reaction between 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) and the thiol group. Concisely, tSH content was measured by mixing 0.15 L of Tris-EDTA buffer, 0.025 L of kidney homogenate samples, 0.79 L of absolute methanol, and 0.010 L of 10 mM DTNB (D8130, Sigma, USA). Eventually, the optical density of the supernatant was read at 412 nm. T-SH level was reported by using a molar absorption coefficient of 13,600 M^{-1} cm^{-1} component (26).

Measurement of nitric oxide metabolite

Nitrite content as an index of nitric oxide (NO) metabolite was determined according to the Griess reaction (33). The level of nitrite was expressed as µmol/g tissue using sodium nitrite as a standard (0-100 µmol/L).

Measurement of antioxidant enzyme activity

Activity levels of antioxidant enzymes, including catalase (CAT) and superoxide dismutase (SOD) in the kidney homogenate tissues were evaluated colorimetrically at the wavelength of 570 nm based on the manufacturer's instructions of assay kits (Karmania Pars Gene, Kerman, Iran) by ELISA reader (Biotek, Netherlands) and presented as U/mL.

Measurement of interleukin-6 and tumor necrosis factor

The serum levels of tumor necrosis factor (TNF-α) and interleukin-6 (IL-6) were determined using an ELISA kit (Karmania Pars Gene, Kerman, Iran).

Measurement of gene expression

The mRNA expression of TNF-α in kidney samples was determined by quantitative real-time polymerase chain reaction (PCR). In short, total RNA was removed from kidney tissue by a total RNA Extraction Kit (Parstous, Mashhad, Iran) based on the producer's guidelines. Complementary DNA (cDNA) was produced using a cDNA Synthesis Kit (Karmania Pars Gene, Kerman, Iran). SYBR Green master mix (Ampliqon, Denmark) was added to cDNA samples and particular primers. The mRNA levels of TNF-α were normalized to β-actin mRNA levels. Results were determined relative to β-actin using the ΔCt^2 method. The following primer pairs were used: forward, 5-TCA GCC TCT TCT CAT TCC TGC -3 and reverse, 5-TTG GTG GTT TGC TAC GAC GTG-3 for TNF-α; forward, 5-GCA AAT GCT TCT AGG CGG AC -3 and reverse, 5-AAG AAA GGG TGT AAA ACG CAG C -3 for β-actin (ACTB).

Histopathological evaluation

Kidney samples were fixed in the buffered 10% formaldehyde. After dehydration through a graded alcohol series, the samples were cleared in xylene. Then, kidney samples were embedded in paraffin (Erma, Japan). Routine staining with hematoxylin and eosin was done for the kidney sections. In a blinded fashion, each section was examined in at least 10 randomly selected non-overlapping fields under a light microscope. In renal tissue, microscopic changes, especially interstitial inflammation, were assessed under a digital research microscope (Olympus BX51, Germany) by a pathologist who was blinded to the groups. The level of each pathological manifestation was graded according to the observed changes as follows: 0, no change; 1, slight change; 2, moderate change; and 3, severe change.

Statistical analysis

Data were presented as mean ± SEM. One-way ANOVA followed by Tukey's post-hoc test was used to compare the differences among groups using GraphPad Prism 8 Software (RRID: SCR_002798). $P \leq 0.05$ was considered statistically significant.

RESULTS

Biochemical parameters

The serum levels of Cr (Fig. 1A) and BUN (Fig. 1B) were notably higher in the EtOH group in comparison to the control group. Pre-treatment with SPB markedly diminished Cr and BUN serum levels in the SPB + EtOH group in comparison to the EtOH group.

Tissue oxidative stress markers

The levels of oxidative stress markers, including MDA (Fig. 2A) and nitrite (Fig. 2B) in the EtOH group showed a significant elevation compared to the control group, while the level of T-SH (Fig. 2C) revealed notable diminishment in the EtOH group in comparison to the control group. SPB hydroethanolic extract pre-treatment in the SPB + EtOH group led to a significant reduction in nitrite and MDA levels compared to the EtOH group. Also, pre-treatment with hydroethanolic extract of SPB in the SPB + EtOH group caused a marked elevation in the kidney level of T-SH in comparison to the EtOH group (Fig. 2).

Antioxidant enzymes

The SOD activity (Fig. 3A) had a considerable reduction in the EtOH group in comparison to the control group. In addition, the SOD activity exhibited a considerable increase in the SPB + EtOH group in comparison to the EtOH group. The CAT activity (Fig. 3B) increased significantly in the EtOH group compared to the control group, and treatment with SPB caused a significant decrease in the SPB + EtOH group compared to the EtOH group.

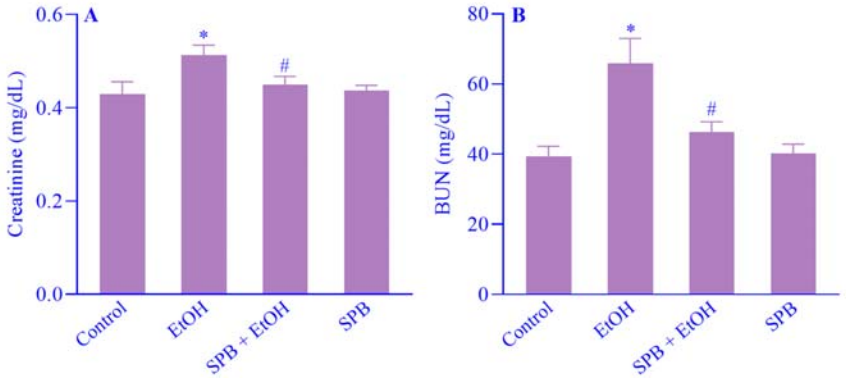

Fig. 1. The effect of SPB hydroethanolic extract on the plasma levels of (A) creatinine and (B) BUN in the EtOH-induced nephrotoxicity in rats. The control group received distilled water as a vehicle. The animals received EtOH and SBP hydroalcoholic extract at doses of 7 mL/kg and 500 mg/kg, respectively. Data were expressed as mean ± SEM, n = 6. One-way ANOVA followed by Tukey post-hoc test. $^{*}P \leq 0.05$ indicates significant difference compared to the control group; $^{\#}P \leq 0.05$ versus EtOH group. EtOH, Ethanol; SBP, *Stachys pilifera* Benth; BUN, blood urea nitrogen.

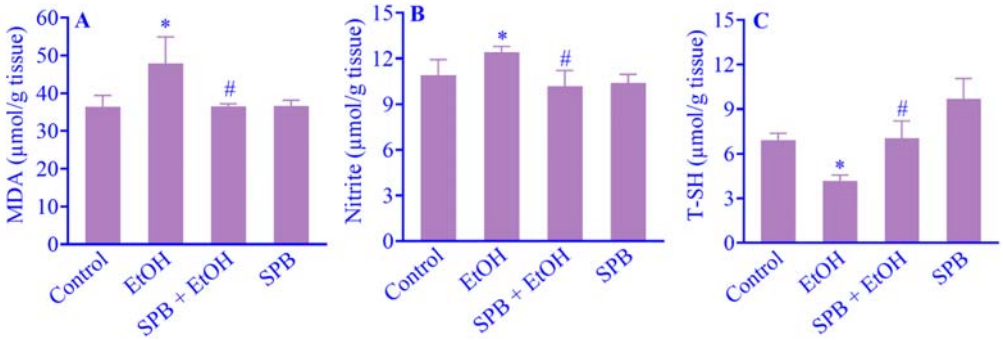

Fig. 2. Effect of hydroalcoholic extract of SPB on tissue levels of (A) MDA, (B) nitrite, and (C) T-SH in the EtOH-induced nephrotoxicity in rats (n = 6). The control group received distilled water as a vehicle. The animals received EtOH and SBP hydroalcoholic extract at doses of 7 mL/kg and 500 mg/kg, respectively. Data were expressed as mean ± SEM, n = 6. One-way ANOVA followed by Tukey post-hoc test. $^{*}P \leq 0.05$ indicates significant difference compared to the control group; $^{\#}P \leq 0.05$ versus EtOH group. EtOH, Ethanol; SBP, *Stachys pilifera* Benth; MDA, malondialdehyde; T-SH, total thiol group.

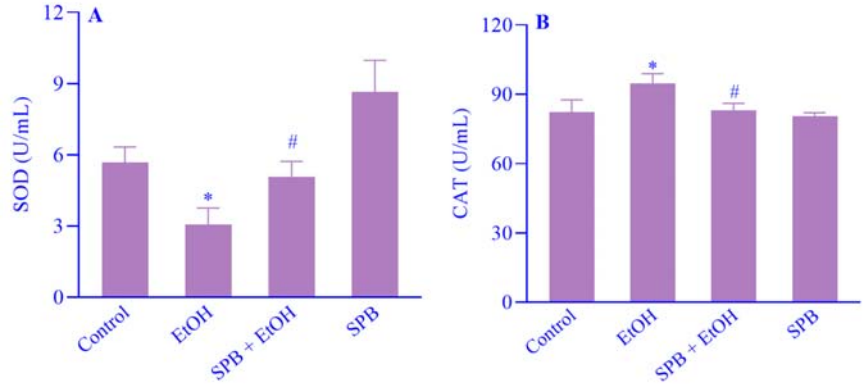

Fig. 3. Effect of hydroalcoholic extract of SPB on the activity of antioxidant enzymes of (A) SOD and (B) CAT in the EtOH-induced nephrotoxicity in rats (n = 6). The control group received distilled water as a vehicle. The animals received EtOH and SBP hydroalcoholic extract at the doses of 7 mL/kg and 500 mg/kg, respectively. Data were expressed as mean ± SEM, n = 6. One-way ANOVA followed by Tukey post-hoc test. $^{*}P \leq 0.05$ indicates significant difference compared to the control group; $^{\#}P \leq 0.05$ versus EtOH group. EtOH, Ethanol; SBP, *Stachys pilifera* Benth. SOD, superoxide dismutase; CAT, catalase.

Pro-inflammatory cytokines

The levels of pro-inflammatory cytokines, including IL-6 (Fig. 4A) and TNF-α (Fig. 4B) in the EtOH group were markedly higher compared to the control group. Pre-treatment with SPB in the SPB + EtOH group led to a significant reduction in the serum levels of the cytokines compared to the EtOH group.

Quantitative real-time PCR

To characterize the effect of SPB extract on TNF-α gene expression in the EtOH group, the level of mRNA was measured by real-time PCR. The results denoted a significant elevation in the transcription of the TNF-α encoding gene in the EtOH group in comparison to the control group. SPB pretreatment in the SPB + EtOH group led to significant downregulation in the expression of the TNF-α encoding gene in comparison to the EtOH group (Fig. 5).

Histopathological studies

There were no abnormalities or histological changes in the kidneys of the control and SBP groups (Fig. 6A and B). In the EtOH group (Fig. 6C), interstitial inflammation (grade 1) was observed. In the SBP + EtOH group (Fig. 6D), interstitial inflammation (grade 1) was observed, but in general, the severity of kidney damage was lower in the SBP + EtOH group than that in the EtOH group (Fig. 6).

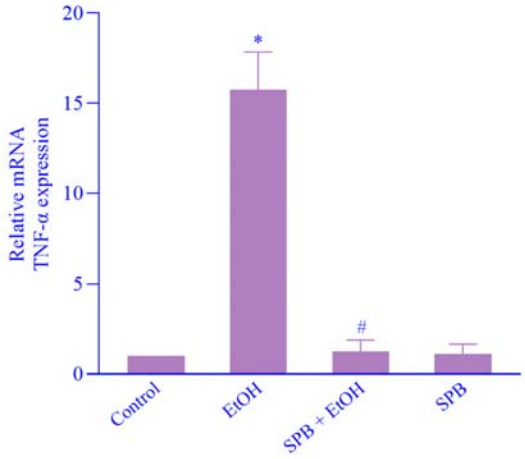

Fig. 5. Effect of hydroalcoholic extract of SPB on the levels of mRNA TNF-α expression in the EtOH-induced nephrotoxicity in rats. The control group received distilled water as a vehicle. The animals received EtOH and SBP hydroalcoholic extract at the doses of 7 mL/kg and 500 mg/kg, respectively. Data were expressed as mean ± SEM, n = 6. One-way ANOVA followed by Tukey post-hoc test. $^{*}P \leq 0.05$ indicates significant difference compared to the control group; $^{\#}P \leq 0.05$ versus EtOH group. EtOH, Ethanol; SBP, *Stachys pilifera* Benth; TNF-α, tumor necrosis factor-α.

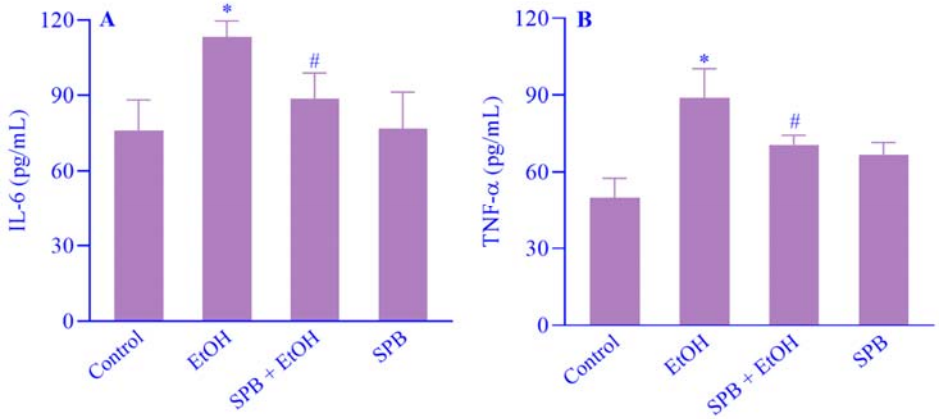

Fig. 4. Effect of hydroalcoholic extract of SPB on the levels of pro-inflammatory cytokines, including (A) IL-6 and (B) TNF-α in the EtOH-induced nephrotoxicity in rats (n = 6). The control group received distilled water as a vehicle. The animals received EtOH and SBP hydroalcoholic extract at doses of 7 mL/kg and 500 mg/kg, respectively. Data were expressed as mean ± SEM, n = 6. One-way ANOVA followed by Tukey post-hoc test. $^{*}P \leq 0.05$ indicates significant difference compared to the control group; $^{\#}P \leq 0.05$ versus EtOH group. EtOH, Ethanol; SBP, *Stachys pilifera* Benth; IL-6, interleukin-6; TNF-α, tumor necrosis factor-alpha.

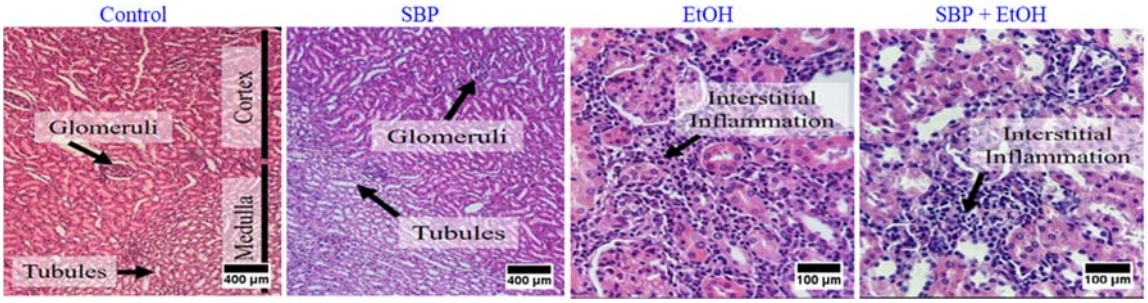

Fig. 6. Representative light microphotographs (hematoxylin and eosin staining) of the kidney obtained from experimental groups (100× magnification for control and SBP groups with scale bar = 400 µm and 400× magnification for EtOH and SBP + EtOH groups with scale bar = 100 µm). The control group received distilled water as a vehicle. The animals received EtOH and SBP hydroalcoholic extract at the doses of 7 mL/kg and 500 mg/kg, respectively. EtOH, Ethanol; SBP, *Stachys pilifera* Benth.

DISCUSSION

Alcohol use disorder (AUD) is a substantial public health problem with the major consequences of chronic AUD that include increased alcohol-related morbidity and mortality, such as liver cirrhosis, liver cancer, pancreatitis, and cardiovascular complications. At present, the epidemiological data linking AUD to an increased risk of chronic renal disease are debatable. However, multiple preclinical investigations demonstrated that alcohol use has a significant impact on the kidney, indicating the possibility of a distinct pathologic entity known as alcoholic kidney injury (7).

Ethanol is mainly metabolized in the liver and generates toxic metabolites such as acetaldehyde, which are excreted through the kidneys into the urine (17,34). Literature has shown that excessive alcohol consumption causes kidney functional damage due to necrosis and disrupts renal filtration (35). In the present study, the administration of ethanol in the EtOH group demonstrated apparent nephrotoxic consequences as determined by the boosted BUN and Cr, as well as histopathological alterations, such as cytoplasmic vacuolation, tubular dilation, tubular cast, interstitial inflammation, and cortical vascular congestion. Studies have shown that chronic ethanol exposure significantly increases plasma creatinine and urea levels (14,35,36). Also, the present results exhibited that pretreatment with SPB extract led to a significant reduction in plasma BUN and Cr levels and improved renal function compared to the EtOH group, which was confirmed by the previous reports (29). Sadeghi's study revealed the nephroprotective effects of SPB in cisplatin-induced kidney damage in rats (29).

It seems that mechanisms such as oxidative stress and inflammatory reactions play notable roles in the ethanol-induced nephrotoxicity (10,14). The mitochondrial and microsomal systems involved in ethanol metabolism are connected to alcohol-induced oxidative stress. The generation of reactive nitrogen species (RNS) and ROS is closely related to the metabolism of ethanol. GSH levels are reduced, and antioxidant activity is reduced when ethanol is used. It increases protein adducts of MDA, hydroxyethyl radical, and hydroxynonenal. These lead to the alteration of all biological structures, which in turn causes severe tissue and cell dysfunction (37).

Alcohol causes hyperacetylation of proteins found in mitochondria in the kidney, which may disrupt the activity of certain mitochondrial proteins associated with alcohol metabolism or safeguard from oxidative stress (such as glutathione peroxidase, aldehyde dehydrogenase 2, and SOD 2) (7,11). Ethanol is metabolized in the kidney by renal CYP2E1, alcohol dehydrogenase, and CYP24A1, which produces ROS in the kidney (16,38). MDA is one of the markers for evaluating oxidative stress and is generated due to lipid peroxidation induced by free radicals (39). The T-SH is another indicator of oxidative stress, and its diminishment in the evaluation of the antioxidant system is one of the significant signs of oxidative stress (29).

Research indicated that ethanol intake may raise the expression of other possible free radical sources in the kidneys, including a group of enzymes known as NO synthase (NOS), promoting the synthesis of NO (40). When NO is produced in excess, it can combine with other molecules and form free radicals, causing renal tissue damage. Ethanol use also boosted endothelial NOS (eNOS) and inducible NOS (iNOS) (40). It is still unknown, though, how precisely ethanol increases NOSs and whether it does so directly or indirectly. Given ethanol's effects on the digestive system, it is possible that toxins produced from the intestines into the bloodstream trigger the production of NOSs. Oxidation or the absence of essential coenzymes (such as tetrahydrobiopterin) may cause both enzymes to uncouple, according to another idea. Uncoupling eventually results in the production of harmful ROS, such as superoxide anion, rather than of the vasorelaxant NO that keeps the kidney blood flow regular (7). Excessive levels of ROS can lead to the production of free radicals, which can harm kidney tissue. Furthermore, the impact of AUD on the liver, heart, intestines, and skeletal muscle might set off harmful pathological processes that harm the kidneys (41,42).

The results of this study showed that ethanol administration induced oxidative kidney injury as verified by a major increase in MDA and nitrite, as well as a diminishment in T-SH levels in the renal tissues of ethanol-administered rats compared to the control group. The results were in concordance with previous studies (40,43-46). Also, the results demonstrated that pretreatment with SPB extract diminished nitrite and MDA levels and increased T-SH levels in kidney homogenate tissues compared to the EtOH group, which were consistent with previous studies (27,29). The antioxidant properties of SPB have been proven in earlier studies (29,47,48).

Cells are protected against damage caused by free radicals in physiological conditions due to having enzymatic and non-enzymatic antioxidant mechanisms (27). SOD and CAT enzymes are a very significant part of the enzyme antioxidant system in the cell, which play a vital role in eradicating free radicals. These antioxidant enzymes work together to vanquish ROS (49). High levels of free radicals reduce cellular antioxidant capacity and disrupt the balance between antioxidants and oxidants. In such a situation, the cell is prone to damage (50,51). The present results exhibited a considerable decrease in enzyme SOD activity in the EtOH group compared to the control group. This reduction indicated the defeat of the SOD pathway against oxidative stress. The SOD activity in alcohol-induced hepato-nephrotoxicity in rats was meaningfully reduced in comparison to the control group (17). Post-unilateral nephrectomy administration of alcohol caused significantly decreased SOD and CAT (43). The findings of this study showed that SPB extract pretreatment significantly increased SOD activity in comparison to the EtOH group, which could indicate the improvement of the cell antioxidant system. Rabani *et al*. reported that the hydroalcoholic extract of SPB increased tissue SOD levels in acetaminophen-induced nephrotoxicity (52). Also, the results of this study exhibited that the level of CAT activity in the EtOH group was notably elevated in comparison to the control group. The finding was consistent with studies that have shown that ethanol increased renal CAT activity levels (6,43). The relative contribution of antioxidant enzymes in hydrogen peroxide elimination was determined by tissue characteristics. In kidney tissue, CAT is the most involved in the decomposition of hydrogen peroxide. An increase in CAT activity in kidney tissue could indicate an increase in renal ethanol tolerance. SPB extract pretreatment led to a significant reduction in renal CAT activity level compared to the EtOH group.

TNF-α, a proinflammatory cytokine, is mostly produced by immune cells like macrophages and can induce inflammatory mediators such as eicosanoids, IL-1, and platelet-activating factors (53). TNF-α is either free in plasma or connected to TNF receptors 1 and 2 in circulation. TNF-α and its receptors are expressed in leukocytes, tubular cells, and glomerular cells during kidney inflammation (54). It has been shown that ethanol consumption causes kidney inflammation and the gathering of inflammatory cells in the renal tissue apoptosis (14). The levels of TNF-α and nuclear factor-κB showed a significant increase

in male Wistar rats treated with ethanol for 6 weeks (54). The current experiment measured IL-6 and TNF-α levels to evaluate the inflammatory status. The findings showed that IL-6 and TNF-α levels in the EtOH group were significantly higher compared to those in the control animals. Also, the results exhibited that pretreatment with SPB extract decreased TNF-α and IL-6 levels in comparison to the EtOH group. Ethanol (20%, v/v) consumption increased IL-6 and TNF-α levels in the C57BL/6J mouse model of sub-lethal sepsis (36). In addition, this study measured TNF-α gene expression in kidney tissue to evaluate the inflammatory state of the kidney. The results exhibited that the content of TNF-α gene expression in the renal tissue of the EtOH group was significantly higher compared to the control animals. A prior study has reported the anti-inflammatory impacts of the SPB (19). In the current study, the oral administration of SPB extract significantly decreased TNF-α gene expression levels in renal tissue compared to the EtOH group.

CONCLUSION

Briefly, the findings showed that ethanol administration led to a reduction in renal function, an increase in tissue oxidative stress, and an elevation in cytokines involved in the inflammation process. However, SPB pretreatment caused nephroprotective, antioxidant, and anti-inflammatory effects against EtOH-induced nephrotoxicity.

Acknowledgments

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Conflict of interest statement

All authors declared no conflict of interest in this study.

Authors' contribution

A. Taheri Mirghaed, N. Khoshkharam, and S. Abdzadeh were responsible for conducting the experiments and writing the first version of the manuscript; A.H. Doustimotlagh, F. Karimi, M. Razazan, and Z. Rahami were responsible for the idea and design of the experiments, writing, reviewing and editing the first draft, and analyzing the data. All authors read and approved the final version of the manuscript.

REFERENCES

1. Molina PE, Gardner JD, Souza-Smith FM, Whitaker AM. Alcohol abuse: critical pathophysiological processes and contribution to disease burden. Physiology (Bethesda). 2014;29(3):203-215. DOI: 10.1152/physiol.00055.2013.
2. Bagnardi V, Rota M, Botteri E, Tramacere I, Islami F, Fedirko V, *et al*. Alcohol consumption and site-specific cancer risk: a comprehensive dose-response meta-analysis. Br J Cancer. 2015;112(3):580-593. DOI: 10.1038/bjc.2014.579.
3. Husain K, Ansari RA, Ferder L. Alcohol-induced hypertension: mechanism and prevention. World J Cardiol. 2014;6(5):245-252. DOI: 10.4330/wjc.v6.i5.245.
4. Arora M, ElSayed A, Beger B, Naidoo P, Shilton T, Jain N, *et al*. The impact of alcohol consumption on cardiovascular health: myths and measures. Glob Heart. 2022;17(1):45,1-11. DOI: 10.5334/gh.1132.
5. Lima F, Sims S, O'Donnell M. Harmful drinking is associated with mental health conditions and other risk behaviours in Australian young people. Aust N Z J Public Health. 2020;44(3):201-217. DOI: 10.1111/1753-6405.12978.
6. Dinu D, Nechifor MT, Movileanu L. Ethanol-induced alterations of the antioxidant defense system in rat kidney. J Biochem Mol Toxicol. 2005;19(6):386-395. DOI: 10.1002/jbt.20101.
7. Varga ZV, Matyas C, Paloczi J, Pacher P. Alcohol misuse and kidney injury: epidemiological evidence and potential mechanisms. Alcohol Res. 2017;38(2):283-288. PMID: 28988579.
8. Lee YJ, Cho S, Kim SR. Effect of alcohol consumption on kidney function: population-based cohort study. Sci Rep. 2021;11(1):2381,1-9. DOI: 10.1038/s41598-021-81777-5.
9. Adewale A, Ifudu O. Kidney injury, fluid, electrolyte and acid-base abnormalities in alcoholics. Niger Med J. 2014;55(2):93-98. DOI: 10.4103/0300-1652.129631.
10. Hebbani AV, Bulle S, Kanu VR, Balachandrababu Malini A, Reddy VD, Chakravarthula VN. Nephro-protective activity of wheatgrass juice against alcohol-induced oxidative damage in rats. Toxicol Mech Methods. 2020;30(9):679-686. DOI: 10.1080/15376516.2020.1810837.
11. Harris PS, Roy SR, Coughlan C, Orlicky DJ, Liang Y, Shearn CT, *et al*. Chronic ethanol consumption induces mitochondrial protein acetylation and oxidative stress in the kidney. Redox Biol. 2015; 6:33-40. DOI: 10.1016/j.redox.2015.06.021.

12. Zhan Z, Chen J, Zhou H, Hong X, Li L, Qin X, *et al*. Chronic alcohol consumption aggravates acute kidney injury through integrin β1/JNK signaling. Redox Biol. 2024;77:103386,1-14. DOI: 10.1016/j.redox.2024.103386.
13. Rodrigo R, Rivera G. Renal damage mediated by oxidative stress: a hypothesis of protective effects of red wine. Free Radic Biol Med. 2002;33(3):409-422. DOI: 10.1016/s0891-5849(02)00908-5.
14. Latchoumycandane C, Nagy LE, McIntyre TM. Chronic ethanol ingestion induces oxidative kidney injury through taurine-inhibitable inflammation. Free Radic Biol Med. 2014;69:403-416. DOI: 10.1016/j.freeradbiomed.2014.01.001.
15. Kubo K, Saito M, Tadokoro T, Maekawa A. Changes in susceptibility of tissues to lipid peroxidation after ingestion of various levels of docosahexaenoic acid and vitamin E. Br J Nutr. 1997;78(4):655-669. DOI: 10.1079/bjn19970181.
16. Ozbek E. Induction of oxidative stress in kidney. Int J Nephrol. 2012;2012:465897,1-9. DOI: 10.1155/2012/465897.
17. El-Megid A, Mohamed H, Abdul Azeem A, El-Shahat A, Mekawey H. Identification of the therapeutic effects of gamma-irradiated chamomile aqueous extract against alcohol induced hepato-nephrotoxicity in rats. Egypt J Rad Sci Applic. 2017;30(1):53-61. DOI: 10.21608/ejrsa.2017.4467.
18. Zhu W, Zhang Y, Huang Y, Lu L. Chinese herbal medicine for the treatment of drug addiction. Int Rev Neurobiol. 2017;135:279-295. DOI: 10.1016/bs.irn.2017.02.013.
19. Sadeghi H, Zarezade V, Sadeghi H, Toori MA, Barmak MJ, Azizi A, *et al*. Anti-inflammatory activity of *Stachys Pilifera* benth. Iran Red Crescent Med J. 2014;16(9):1-8. DOI: 10.5812/ircmj.19259.
20. Panahi Kokhdan E, Ahmadi K, Sadeghi H, Sadeghi H, Dadgary F, Danaei N, *et al*. Hepatoprotective effect of *Stachys pilifera* ethanol extract in carbon tetrachloride-induce hepatotoxicity in rats. Pharm Biol. 2017;55(1):1389-1393. DOI: 10.1080/13880209.2017.1302484.
21. Zhu S, Pabla N, Tang C, He L, Dong Z. DNA damage response in cisplatin-induced nephrotoxicity. Arch Toxicol. 2015;89(12):2197-2205. DOI: 10.1007/s00204-015-1633-3.
22. Mazer M, Perrone J. Acetaminophen-induced nephrotoxicity: pathophysiology, clinical manifestations, and management. J Med Toxicol. 2008;4(1):2-6. DOI: 10.1007/BF03160941.
23. Granata S, Votrico V, Spadaccino F, Catalano V, Netti GS, Ranieri E, *et al*. Oxidative stress and ischemia/reperfusion injury in kidney transplantation: focus on ferroptosis, mitophagy and new antioxidants. Antioxidants (Basel). 2022;11(4):796,1-17. DOI: 10.3390/antiox11040769.
24. McSweeney KR, Gadanec LK, Qaradakhi T, Ali BA, Zulli A, Apostolopoulos V. Mechanisms of cisplatin-induced acute kidney injury: pathological mechanisms, pharmacological interventions, and genetic mitigations. Cancers. 2021;13(7):1572,1-42. DOI: 10.3390/cancers13071572.
25. Güler MC, Tanyeli A, Akdemir FNE, Eraslan E, Şebin SÖ, Erdoğan DG, *et al*. An overview of ischemia-reperfusion injury: review on oxidative stress and inflammatory response. Eurasian J Med. 2022;54(Suppl 1):S62-S65. DOI: 10.5152/eurasianjmed.2022.22293.
26. Azarmehr N, Afshar P, Moradi M, Sadeghi H, Sadeghi H, Alipoor B, *et al*. Hepatoprotective and antioxidant activity of watercress extract on acetaminophen-induced hepatotoxicity in rats. Heliyon. 2019;5(7). e02072,1-5. DOI: 10.1016/j.heliyon.2019.e02072.
27. Mansourian M, Mirzaei A, Azarmehr N, Vakilpour H, Kokhdan EP, Doustimotlagh AH. Hepatoprotective and antioxidant activity of hydroalcoholic extract of *Stachys pilifera* Benth on acetaminophen-induced liver toxicity in male rats. Heliyon. 2019;5(12):1-5. DOI: 10.1016/j.heliyon.2019.e03029.
28. Pal LC, Agrawal S, Gautam A, Chauhan JK, Rao CV. Hepatoprotective and antioxidant potential of phenolics-enriched fraction of *Anogeissus acuminata* leaf against alcohol-induced hepatotoxicity in rats. Med Sci (Basel). 2022;10(1):17,1-14. DOI: 10.3390/medsci10010017.
29. Sadeghi H, Mansourian M, Panahi Kokhdan E, Salehpour Z, Sadati I, Abbaszadeh-Goudarzi K, *et al*. Antioxidant and protective effect of *Stachys pilifera* Benth against nephrotoxicity induced by cisplatin in rats. J Food Biochem. 2020;44(5):e13190,1-10. DOI: 10.1111/jfbc.13190.
30. Karimi F, Nematbakhsh M. Renal vascular responses to angiotensin II infusion in two kidneys-one clip hypertensive rats under partial ischemia/reperfusion with and without ischemia preconditioning: the roles of AT1R blockade and co-blockades of AT1R and MasR. Res Pharm Sci. 2023;18(4):392-403. DOI: 10.4103/1735-5362.378086.
31. Mas-Bargues C, Escrivá C, Dromant M, Borrás C, Viña J. Lipid peroxidation as measured by chromatographic determination of malondialdehyde. Human plasma reference values in health and disease. Arch Biochem Biophys. 2021;709:108941,1-7. DOI: 10.1016/j.abb.2021.108941.
32. Akbari G, Abasi MR, Gharaghani M, Nouripoor S, Shakerinasab N, Azizi M, *et al*. Antioxidant and hepatoprotective activities of *Juniperus excelsa M. Bieb* against bile duct ligation-induced cholestasis. Res Pharm Sci. 2024;19(2):217-227. DOI: 10.4103/RPS.RPS-52-23.
33. Sadeghi H, Jahanbazi F, Sadeghi H, Omidifar N, Alipoor B, Kokhdan EP, *et al*. Metformin attenuates oxidative stress and liver damage after bile duct ligation in rats. Res Pharm Sci. 2019;14(2): 122-129. DOI: 10.4103/1735-5362.253359.
34. Yu HS, Oyama T, Isse T, Kitagawa K, Tanaka M, Kawamoto T. Formation of acetaldehyde-derived

DNA adducts due to alcohol exposure. Chem Biol Interact. 2010;188(3):367-375.
DOI: 10.1016/j.cbi.2010.08.005.
35. Samadi M, Shirpoor A, Afshari AT, Kheradmand F, Rasmi Y, Sadeghzadeh M. Chronic ethanol ingestion induces glomerular filtration barrier proteins genes expression alteration and increases matrix metalloproteinases activity in the kidney of rats. Interv Med Appl Sci. 2018;10(3):171-177.
DOI: 10.1556/1646.10.2018.23.
36. Do Valle GT, Ricci ST, Silva AO, Tirapelli CR, Ceron CS. Ethanol consumption increases renal dysfunction and mortality in a mice model of sub-lethal sepsis. Can J Physiol Pharmacol. 2021;99(7):699-707.
DOI: 10.1139/cjpp-2020-0564.
37. Das SK, Vasudevan D. Alcohol-induced oxidative stress. Life Sci. 2007;81(3):177-187.
DOI: 10.1016/j.lfs.2007.05.005.
38. Shankar K, Liu X, Singhal R, Chen JR, Nagarajan S, Badger TM, *et al*. Chronic ethanol consumption leads to disruption of vitamin D3 homeostasis associated with induction of renal 1, 25 dihydroxyvitamin D3-24-hydroxylase (CYP24A1). Endocrinology. 2008;149(4):1748-1756.
DOI: 10.1210/en.2007-0903.
39. Cederbaum AI, Lu Y, Wu D. Role of oxidative stress in alcohol-induced liver injury. Arch Toxicol. 2009;83(6):519-548.
DOI: 10.1007/s00204-009-0432-0.
40. Tirapelli LF, Martins-Oliveira A, Batalhão ME, Tirapelli DP, Carnio EC, Tanus-Santos JE, *et al*. Ethanol consumption increases the expression of endothelial nitric oxide synthase, inducible nitric oxide synthase and metalloproteinases in the rat kidney. J Pharm Pharmacol. 2012;64(1):68-76.
DOI: 10.1111/j.2042-7158.2011.01396.x.
41. Okan A, Öztürk O, Yılmaz S, Doğanyiğit Z, Uguz AC, Eroglu E. Immunoreactivity of NOS2 and NF-? B in kidney tissue in experimental alcohol consumption model. Ank Üniverc Fac. 2023;76(1):17-23.
DOI: 10.4274/atfm.galenos.2023.46873.
42. Toda N, Ayajiki K. Vascular actions of nitric oxide as affected by exposure to alcohol. Alcohol Alcohol. 2010;45(4):347-355.
DOI: 10.1093/alcalc/agq028.
43. Onwuka O, Abiye P, Ajuzie G. Post-unilateral nephrectomy administration of alcohol escalates kidney oxidative stress of male Wistar rats. J Appl Sci Environ Manag. 2023;27(11):2575-2580.
DOI: 10.4314/jasem.v27i11.29.
44. Abarikwu SO, Duru QC, Njoku RC, Amadi BA, Tamunoibuomie A, Keboh E. Effects of co-exposure to atrazine and ethanol on the oxidative damage of kidney and liver in Wistar rats. Ren Fail. 2017;39(1):588-596.
DOI: 10.1080/0886022x.2017.1351373.
45. Cigremis Y, Turkoz Y, Akgoz M, Sozmen M. The effects of chronic exposure to ethanol and cigarette smoke on the level of reduced glutathione and malondialdehyde in rat kidney. Urol Res. 2004;32(3):213-218.
DOI: 10.1007/s00240-004-0406-x.
46. da Silva CB, Ceron CS, Mendes AS, de Martinis BS, Castro MM, Tirapelli CR. Inducible nitric oxide synthase (iNOS) mediates ethanol-induced redox imbalance and upregulation of inflammatory cytokines in the kidney. Can J Physiol Pharmacol. 2021;99(10):1016-1025.
DOI: 10.1139/cjpp-2021-0108.
47. Farjam MH, Khalili M, Rustayian A, Javidnia K, Izadi S. Biological activity of the n-butanolic extract of *Stachys pilifera*. Afr J Microbiol Res. 2011;5(28):5115-5119.
DOI: 10.5897/AJMR11.1066.
48. Khademian A, Tabefam M, Mazarei Z, Kanani MR, Sepehri H, Delphi L, *et al*. Chemical constituents and cytotoxic activity of *Stachys pilifera* Benth. S Afr J Bot. 2021;139:226-229.
DOI: 10.1016/j.sajb.2021.02.022.
49. Nagi MN, Almakki HA. Thymoquinone supplementation induces quinone reductase and glutathione transferase in mice liver: possible role in protection against chemical carcinogenesis and toxicity. Phytother Res. 2009;23(9):1295-1298.
DOI: 10.1002/ptr.2766.
50. Ince S, Kucukkurt I, Cigerci IH, Fatih Fidan A, Eryavuz A. The effects of dietary boric acid and borax supplementation on lipid peroxidation, antioxidant activity, and DNA damage in rats. J Trace Elem Med Biol. 2010;24(3):161-164.
DOI: 10.1016/j.jtemb.2010.01.003.
51. Mani V, Arivalagan S, Siddique AI, Namasivayam N. Antioxidant and anti-inflammatory role of zingerone in ethanol-induced hepatotoxicity. Mol Cell Biochem. 2016;421(1-2):169-181.
DOI: 10.1007/s11010-016-2798-7.
52. Rabani MR, Azarmehr N, Moslemi Z, Sadeghi H, Amini-Khoei H, Doustimotlagh AH. Protective effects of hydroalcoholic extract of S*tachys pilifera* on paracetamol-induced nephrotoxicity in female rats. Res Pharm Sci. 2021;16(6):643-650.
DOI: 10.4103/1735-5362.327510.
53. Laha D, Grant R, Mishra P, Nilubol N. The role of tumor necrosis factor in manipulating the immunological response of tumor microenvironment. Front Immunol. 2021;12:656908,1-12.
DOI: 10.3389/fimmu.2021.656908.
54. Abdollahzade Fard A, Samadi M, Shirpoor A, Rasmi Y. Ethanol consumption promotes TNF-α signaling pathway in rat kidney: rescue effect of curcumin. J Chem Health Risks. 2022;12(2): 271-280.
DOI: 10.22034/jchr.2021.1913474.1211.