Aqueous Myrrh Extract Relieves Oxidative Stress-Dependent Nephrotoxicity Induced by Ethanol in Male Rats

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ABSTRACT

The kidney is one of the target organs whose structure and functions are significantly influenced by toxins. Because the protective impact of myrrh resin on renal damage induced by alcohol intake has not been previously evaluated, this study was designed to investigate the in vivo antioxidant activity of aqueous myrrh extract (AME) on oxidative stress-dependent nephrotoxicity caused by ethanol in rats. Male Sprague–Dawley rats orally received 40% ethanol (3 g kg^{-1}) and were then orally treated with (500 mg kg^{-1}) AME daily for 30 days. Kidney function, oxidant and antioxidant status, and histopathology alterations in renal tissue were measured. Animals that received ethanol displayed nephrotoxicity, as confirmed by markedly increased serum creatinine, urea, and uric acid levels along with histological changes, including shrunken glomeruli, necrosis, inflammatory cell infiltration, congestion, and edema. They also exhibited significantly elevated lipid peroxidation and oxidant radicals with decreased antioxidant parameters in renal tissue. In contrast, AME alone did not cause renal injury, and it was able to notably improve the toxic impacts of ethanol in the kidney when administered together. In conclusion, AME provided preservation against ethanol toxicity by scavenging reactive oxygen species (ROS) and relieving oxidative stress as a result of its rich chemical content of furano-sesquiterpenes, which possess powerful antioxidant activities.

INTRODUCTION

The kidney is one of the body's most significant excretory organs, and it is where the majority of harmful compounds can accumulate (Thangapandiyan *et al*., 2019). It is often involved in harmful effects induced by exposure to foreign substances due to direct toxicity or indirect toxicity through the deposition of crystals or damage to the blood vasculature in renal tissue (Faria *et al*., 2019). The process of renal function failure can be accelerated by a variety of factors, including diabetes, hypertension, and some drugs (Bundy *et al*., 2018).

Alcohol consumption has serious toxic effects on body health and causes more than 200 diseases worldwide

(Rungratanawanich *et al*., 2021). Although various organs may be injured by inflammatory conditions and oxidative stress caused by heavy alcohol consumption, the kidneys are one of the main organs directly affected by alcohol toxicity (Sousa *et al*., 2021). At the initial stage of alcohol cytotoxicity, alcohol causes hyperacetylation of renal mitochondrial proteins, which results in kidney impairment through the disrupted metabolic pathway and impaired antioxidant system (Harris *et al*., 2015). Several studies have reported that ethanol intake can attenuate kidney function and renal reabsorption, and it has also been linked to increased cellular proliferation and inflammation in the renal tubules, which results in the aberrant expansion of the glomerular basement membrane (Cikler-Dulger and Sogut, 2020; Lee *et al*., 2021). Due to these negative impacts, the kidney's capacity to control the body's fluid volume and electrolyte balance is compromised (Varga *et al*., 2017).

Myrrh is a resinous exudate produced by plants known as Nees that belong to the species *Commiphora myrrha* (Latha *et al*., 2021), which is native to India, Africa, and southern Arabia (Suleiman, 2015). It is one of the oldest herbal medicines widely recognized to have been employed by ancient cultures to preserve

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mummies in Egypt, treat skin irritation in China, and treat chest illnesses in India, and the Indians also used it as a spice in cooking (Massoud *et al.*, 2001). Recent scientific studies have reported that myrrh has a variety of pharmacological benefits as an antitumor, antimicrobial, antifungal, and immunoregulatory drug (Suliman *et al.*, 2022). Various chemical components, including steroids, triterpenes, sesquiterpenes, monoterpenes, and lignans, have been isolated from myrrh. These health-enhancing pharmacological substances give myrrh several curative features, such as antioxidant, anticancer, antimicrobial, anti-inflammatory, and immunostimulant properties (Alqahtani *et al.*, 2020). In addition, several studies on rats have revealed that myrrh could even promote the synthesis of cellular antioxidant potential, which results in a reduction in toxicity symptoms in some soft organs, such as the liver and stomach (Lebda *et al.*, 2021; Alahmari *et al.*, 2022).

Myrrh appears to have significant economic and ecological value based on the above characteristics, and it may be able to be applied in the management of oxidative stress and inflammation-related conditions and toxicities. Although the impacts of several natural products have been widely studied to assess their therapeutic effects against alcohol consumption that causes renal toxicity, this study is aimed to reveal the effectiveness of myrrh extract on the nephrotoxicity induced by ethanol. In this study, we examined whether myrrh could relieve oxidative stress-dependent nephrotoxicity induced by ethanol. We also investigated its efficacy in restoring renal structure and function.

MATERIALS AND METHODS

Plant material and preparation of aqueous extraction

Ethanol and other chemicals were supplied by the Biology Department at King Khalid University, Abha. Myrrh resin was obtained from the herbal store in Khamis Mushait, Saudi Arabia. Its initial source is Saudi Arabia's southern region and comes from *C. myrrha*. It was washed, air-dried, and ground finely before use in aqueous extraction preparation.

Approximately 50 g of ground myrrh was mixed well with 500 mL of distilled water at 80 °C for 10 hours and then centrifuged for 20 min at 4,000 rpm (El-Sherbiny *et al.*, 2013). The aqueous myrrh extract was filtered by a double layer of gauze and kept in a refrigerator until use.

Analysis of the phytochemical contents of myrrh extract using gas chromatography–mass spectrometry (GC–MS)

We previously analyzed the phytochemical compositions of myrrh extract using GC–MS. Curzerene

was the dominant ingredient in the extract in addition to other components, including aromadendrene 2, gamma-elemene, and β-elemene, resulting in a high concentration of sesquiterpenes (Alahmari *et al.*, 2022).

Experimental animals and treatment

Twenty-eight adult male Sprague–Dawley rats (supplied by the Animal House of the Science College at King Khalid University, Abha, Saudi Arabia), approximately 150–250 g in weight, were used in this study. Before performing experiments, the rats were acclimatized under standard laboratory conditions of humidity (45±5%) and temperature (22±2 °C) with 12 h light-dark cycles for 14 days with free access to water and normal food.

The experimental rats were randomly categorized into four groups with seven animals in each group. The first group (CTR) received normal water and diet; the second group (EtOH) received 40% ethanol (3 g kg^{-1}) and was fed a normal diet; the third group (AME) received only aqueous myrrh extract (500 mg kg^{-1}) and was fed a normal diet; and the fourth group (EtOH + AME) received 40% ethanol and was fed a normal diet, and after 2 h, the rats were treated with aqueous myrrh extract at the same dosage as the third group. The treatments were given by gavage daily for 30 days. The doses of EtOH and AME were chosen according to previous studies (Hosseini *et al.*, 2017; Alqahtani *et al.*, 2021).

Sample collection and preparation of kidney homogenate

Twenty-four hours after the end of the dosage, all rats were sacrificed under anesthesia. Blood was collected from the heart puncture, and the serum was centrifuged at 1200× g for 20 min and stored at –20 °C. The kidneys were collected and washed with 0.9% physiological saline for biochemical and histopathological analysis. To prepare kidney tissue homogenate, 1 g of kidney tissue for every 10 mL of phosphate buffer (pH 7.4) was homogenized and centrifuged at 5000× g at 4 °C for 5 min, and then the supernatants were collected and stored at −80 °C for further biochemical analysis (Longobardi *et al.*, 2021).

Assessment of kidney function

The serum levels of kidney functional biomarkers (creatinine, urea, and uric acid) were estimated using colorimetric assay kits from Spectrum Diagnostics (Cairo, Egypt) following the manufacturer's protocols.

Nitric oxide determination

The content of nitric oxide (NO) in the kidney homogenates was estimated using a colorimetric assay kit from Abcam (Cambridge, UK) according to the manufacturer's instructions. In this assay, total nitrate/

nitrite was measured in two steps. First, nitrate was converted into nitrite via nitrate reductase. Second, Griess reagents were utilized to convert nitrite into a deep purple azo compound, the color of which reflected the quantity of nitric oxide in renal tissue.

Hydrogen peroxide assay

To detect changes in free radicals in the renal tissue, the hydrogen peroxide (H_2O_2) level was measured as an indicator of intracellular oxidative stress using diagnostic kits (Spectrum Diagnostics, Cairo, Egypt) following the manufacturer's procedures.

Assessment of renal lipid peroxidation

Lipid peroxidation in renal tissue was estimated by measuring the concentration of malondialdehyde (MDA) using a competitive ELISA kit from Abcam (Cambridge, UK) according to the manufacturer's protocols. Before using the kit, multiple dilutions of renal tissue were carried out to ensure that the readings were within the standard value range.

Assessment of renal antioxidant status

The total antioxidant capacity (TAC) and the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and reduced glutathione (GSH) were evaluated using commercial diagnostic kits from Spectrum Diagnostics (Cairo, Egypt) according to the manufacturer's protocols. The optical density (OD) of the renal supernatants was read by a spectrophotometer at a wavelength of 450 nm.

Cell examination of kidney tissues

Dissected kidney samples were washed in normal saline and then immediately fixed in 10% formalin for 72 h. Then, the samples were dehydrated in a graded series of alcohol and processed twice using xylene. Then, the samples were placed in liquefied paraffin wax and embedded in paraffin. Using a rotary microtome, each sample was cut into 5 µm thick sections. To examine the kidney structure and histopathological lesions in renal tissue, sections were stained with hematoxylin and eosin and Masson's trichrome and then scanned under a digital light microscope (Olympus, Japan). The renal lesions, including degeneration, infiltration, congestion, and edema, were examined and scored in 5 random fields for each slide as follows: score -, absent; score +, mild; score ++, moderate; and score +++, severe, as described previously (Hassanzadeh-Taheri *et al*., 2018). The score of interstitial fibrosis in renal tissue was graded in sections stained with Masson's trichrome as follows: score 0, none; score 1, < 25% of the area; score 2, 25-50% of the area;

and score 3, > 50% of the area, according to previous research (Wu *et al*., 2016).

Statistical analysis

The data are expressed as the mean ± standard error (SE) and were analyzed by one-way analysis of variance (ANOVA) using SPSS statistics software (Version 16). To analyze the significant differences, Tukey's test was performed at $p > 0.05$. In addition, the semiquantitative estimation of the renal fibrosis score was compared among the groups using the Kruskal–Wallis test at $p > 0.05$.

RESULTS

Kidney function biomarkers

The results in Table I illustrate that serum creatinine, urea, and uric acid levels in the EtOH-treated rats were significantly higher ($p > 0.0001$) than those in the CTR rats. In contrast, administration of a mixture of EtOH and AME caused a remarkable reduction ($p > 0.0001$) in both creatinine and urea levels compared to the EtOH group, while there was no significant change ($p > 0.05$) in the level of uric acid under these conditions. On the other hand, the levels of all of the above parameters after treatment with AME alone were similar to those in the CTR group ($p > 0.5$).

NO production in renal tissue

Table I shows the findings regarding the concentration of NO produced in renal tissue. The NO concentration in the kidney was significantly increased in EtOH-treated rats ($p > 0.0001$) compared to the CTR group. However, cotreatment with AME significantly inhibited ($p > 0.05$) the high production of NO induced by EtOH. AME-only treatment did not cause a significant impact on NO production compared to the CTR group.

H_2O_2 level in renal tissue

As shown in Table I, the H_2O_2 level was measured in renal tissues obtained from the CTR and other experimental groups. With respect to the CTR group, the renal H_2O_2 level in the EtOH-treated rats was significantly elevated ($p > 0.05$). Cotreatment with AME was not able to inhibit the release of H_2O_2 ($p > 0.05$) compared to treatment with EtOH alone. There was no significant change ($p > 0.05$) in the level of H_2O_2 between the CTR and AME-treated groups.

Lipid peroxidation and antioxidant status

In the current study, the MDA level in renal tissue was examined as a marker of lipid peroxidation (Table I).

Table I. Effect of myrrh extract administered for 30 days on the values of kidney function parameters (creatinine, urea, and uric acid), nitric oxide (NO), hydrogen peroxide (H_2O_2), and malondialdehyde (MDA). CTR, control; EtOH, 40% ethanol (3 g kg⁻¹); AME, aqueous myrrh extract (500 mg kg⁻¹); and EtOH + AME, 40% ethanol followed by aqueous myrrh extract.

Parameters	Study groups			
	CTR	EtOH	AME	EtOH + AME
Creatinine (mg/dL)	0.83 ± 0.04	$1.31 \pm 0.06^{****}$	0.83 ± 0.03	$0.94 \pm 0.07^{####}$
Urea (mg/dL)	41.77 ± 1.11	$68.62 \pm 2.69^{****}$	40.96 ± 1.05	$52.97 \pm 2.96^{####}$
Uric acid (mg/dL)	1.14 ± 0.07	$1.79 \pm 0.12^{****}$	1.17 ± 0.07	1.52 ± 0.03
NO (μmol/mg)	86.78 ± 4.92	$129.48 \pm 6.11^{****}$	85.16 ± 4.50	$106.64 \pm 5.63^{#}$
H_2O_2 (mM/g)	3.53 ± 0.35	$5.31 \pm 0.44^{*}$	3.53 ± 0.53	5.53 ± 0.20
MDA (nmol/g)	38.79 ± 2.06	$69.92 \pm 3.51^{****}$	34.94 ± 2.50	$57.45 \pm 3.29^{#}$

All values are expressed as the mean ± standard error (SE) of n=7. $^{*}p > 0.05$ and $^{****}p > 0.0001$ *vs.* CTR; $^{#}p > 0.05$ and $^{####}p > 0.0001$ *vs.* EtOH.

Table II. Effect of myrrh extract administered for 30 days on the renal antioxidant capacity (TAC) and the activities of antioxidant parameters in kidney tissues. CTR, control; EtOH, 40% ethanol (3 g kg⁻¹); AME, aqueous myrrh extract (500 mg kg⁻¹); and EtOH + AME, 40% ethanol followed by aqueous myrrh extract. SOD, superoxide dismutase; CAT, catalase; GSH, reduced glutathione; GPx, glutathione peroxidase.

Parameters	Study groups			
	CTR	EtOH	AME	EtOH + AME
TAC (μM/g)	32.72 ± 2.04	$26.49 \pm 1.85^{*}$	33.31 ± 1.50	$30.27 \pm 1.15^{#}$
SOD (U/g)	46.73 ± 2.37	$33.70 \pm 2.42^{**}$	47.25 ± 2.88	$40.73 \pm 1.90^{#}$
CAT (U/g)	7.94 ± 0.33	$5.62 \pm 0.56^{**}$	8.11 ± 0.37	$6.10 \pm 0.29^{#}$
GSH (mg/g)	17.82 ± 0.75	$11.85 \pm 0.46^{****}$	17.22 ± 0.66	$13.01 \pm 0.86^{#}$
GPx (U/g)	14.69 ± 0.73	$11.55 \pm 0.57^{**}$	14.65 ± 0.47	$13.11 \pm 0.63^{#}$

All values are expressed as the mean ± standard error (SE) of n=7. $^{*}p > 0.05$, $^{**}p > 0.01$, and $^{****}p > 0.0001$ *vs.* CTR; $^{#}p > 0.05$ *vs.* EtOH.

In EtOH-treated rats, a significant elevation of renal MDA level was observed ($p > 0.0001$) compared to the CTR rats. Compared to EtOH treatment alone, cotreatment with AME significantly modulated the renal MDA level ($p > 0.05$). On the other hand, administration of AME alone did not exhibit a significant impact on the MDA level when compared with the CTR group.

Table II illustrates the activity of antioxidant parameters in the kidney after 30 days of treatment. There was a noticeable decline in TAC as well as the activities of SOD, CAT, GSH, and GPx after treatment with EtOH alone ($p > 0.05$, $p > 0.01$, $p > 0.01$, $p > 0.0001$, and $p > 0.01$, respectively) compared to those of the CTR group. The values of all parameters (TAC, SOD, CAT, GSH, and GPx) were significantly restored after adding AME to EtOH ($p > 0.05$) compared with EtOH only. No significant differences were observed in the activities of the previous parameters between the CTR and AME groups ($p > 0.05$).

Histopathological examination

The H&E-stained histological sections of renal tissues obtained from both CRT- and AME-treated rats showed integrity of the renal capsule and normal histological architecture of the cortex, which formed the Malpighian renal corpuscle (glomeruli and Bowman's space), proximal convoluted tubules with narrow lumina, distal convoluted tubules with wider lumina, and interlobular blood vessels (Fig. 1A, C). The renal medulla revealed the intact histological structure of the collecting tubules that appeared lined with simple cuboidal epithelium (Fig. 1B, D).

In contrast, treatment with EtOH for 30 days at a dose of (3 g kg⁻¹) caused severe histopathological alterations in renal tissue, including shrunken glomeruli with widened Bowman's space, dilation of tubular lumens, glomerular and tubular congestion, inflammatory infiltration, tubular cell necrosis, interstitial edema, expansion of interlobular veins, and the presence of pyknotic nuclei in most of the

epithelium lining the tubules (Fig. 2A, D). According to the lesion score (Table III), the EtOH-treated group had higher necrosis, infiltration, congestion, and edema than the control group. However, these pathological impacts were largely reduced in rats that received AME after ethanol intake, as shown in Figure 2E and f and Table III for the histopathological scores of the renal lesions.
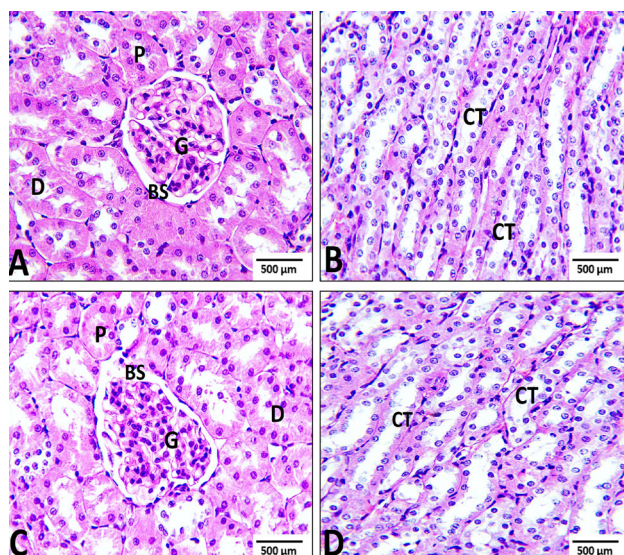

Fig. 1. Effect of aqueous myrrh extract (AME) on histological structure of rat kidney. A, Renal cortex of CTR rats showing normal Malpighian corpuscle containing the glomerulus (G) and Bowman's space (BS), proximal convoluted tubules (P), and distal convoluted tubules (D). B, Renal medulla of CTR rats showing normal collecting tubules (CT). C and D, Renal sections of rats treated with AME only showed no significant alterations in the structure of the renal cortex (C) and medulla (D). Scale bar =500 μm; Stain: H & E. Magnification: 400 ×.

Table III. The histopathological score of the renal lesion after 30 days of treatment. CTR, control; EtOH, 40% ethanol (3 g kg⁻¹); AME, aqueous myrrh extract (500 mg kg⁻¹); and EtOH + AME, 40% ethanol followed by aqueous myrrh extract.

Lesion score	Study groups			
	CTR	EtOH	AME	EtOH + AME
Necrosis	-	+++	-	++
Infiltration	-	+++	+	+
Congestion	-	+++	-	++
Edema	-	+++	-	+

-, absent; +, mild; ++, moderate; +++, severe.

Regarding Masson's trichrome-stained sections (Fig. 3) and the interstitial fibrosis score (Fig. 4) for renal tissue, there was extensive collagen (blue) deposition between the renal tubules and around the interlobular vein (IV) and artery (IA) after being treated with EtOH (Fig. 3C, E) compared to the normal distribution of collagen in the control and AME groups ($p > 0.0001$), which appeared as fine collagen fibers in the corpuscle and between the renal tubules (Fig. 3A, B). The amount of blue collagen fiber deposition significantly declined ($p > 0.001$) after cotreatment with AME for 30 days (Fig. 3F).
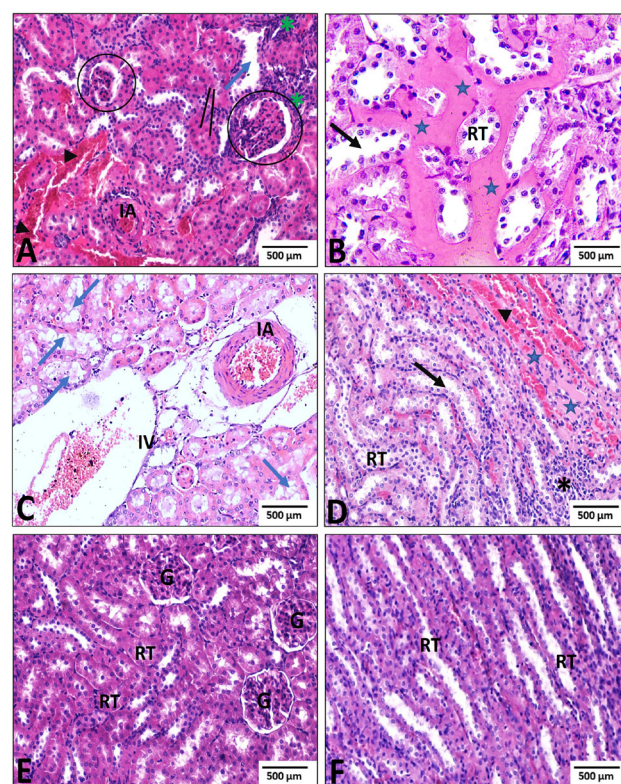

Fig. 2. Effect of aqueous myrrh extract (AME) on histological structure of Ethanol (EtOH) and EtOH + AME-treated groups. A–D, Renal tissue of rats from the EtOH-treated group showing shrunken glomeruli (circle), inflammatory infiltration (*), tubular cell necrosis with vacuolization (blue arrow), dilation of tubular lumens (black arrow), interstitial edema (star), tubular congestion (arrowhead), pyknotic nuclei in most epithelium lining the tubules (line), and expansion of interlobular veins (IV). E and F, Renal sections of rats treated with EtOH + AME showing improved histopathological changes in the renal cortex (E) and medulla (F). G, glomeruli; RT, renal tubule. Scale bar =500 μm; Stain: H & E. Magnification: 200 and 400 ×.
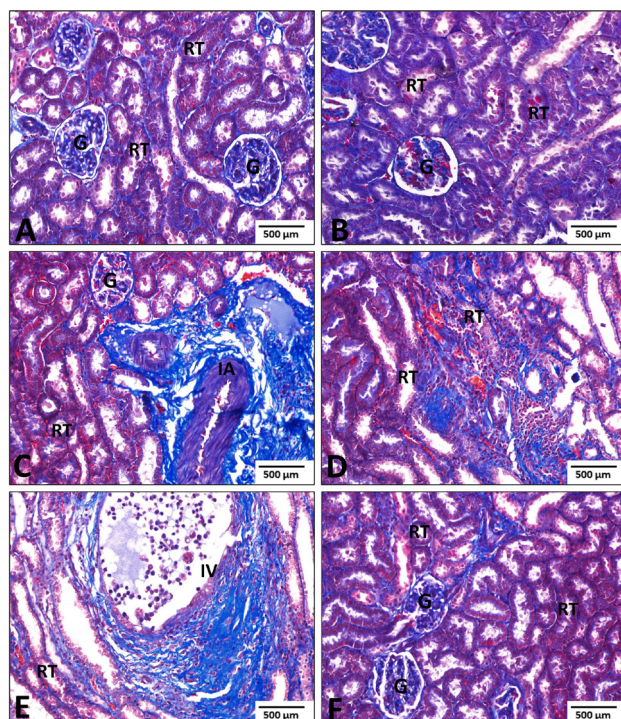
Fig. 3. Images of Masson's trichrome-stained sections of renal tissues from the CTR and experimental groups. A and B, the renal tissues of CTR and AME-treated rats showed a normal distribution of collagen (blue) in the glomeruli (G) and between renal tubules (RT). C-E, the renal tissue of EtOH-treated rats showed a high deposition of collagen fibers (blue) between the renal tubules and around the interlobular vein (IV) and artery (IA). F, the renal tissue of EtOH + AME-treated rats showed mild deposition of collagen fibers (blue) in the glomeruli (G) and between renal tubules (RT). Scale bar =500 μm; 200 ×.
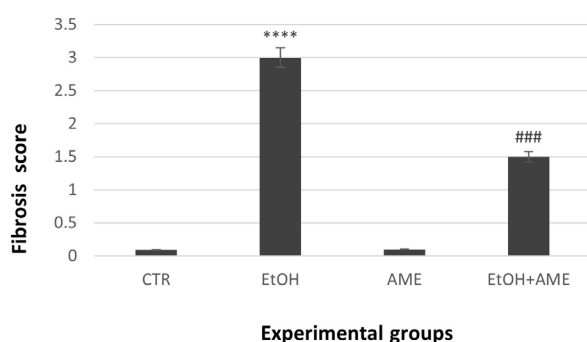

Fig. 4. Semiquantitative estimation of renal fibrosis scores in the experimental groups. All values are expressed as the mean ± standard error (SE). CTR, control; EtOH, 40% ethanol (3 g kg^{-1}); AME, aqueous myrrh extract (500 mg kg^{-1}); and EtOH + AME, 40% ethanol followed by aqueous myrrh extract. *$p > 0.05$ and ****$p > 0.0001$ *vs.* CTR; ###$p > 0.001$ *vs.* EtOH.

DISCUSSION

According to previous experimental studies on animal models, various natural antioxidants have been shown to reduce oxidative nephrotoxicity caused by ethanol (Ramudu *et al*., 2011; Hebbani *et al*., 2020). To date, the ability of myrrh to prevent alcohol-induced nephrotoxicity is still unknown. Therefore, this research aimed to explore the protective impact of myrrh extract on ethanol-induced kidney injury and oxidative stress in adult rats.

Several scientific studies have illustrated that EtOH is harmful to the kidney and causes renal dysfunction (Tirapelli *et al*., 2012). Confirming these studies, we found significantly elevated concentrations of serum creatinine, urea, and uric acid in rats treated with EtOH. These parameters are frequently used as indicators of renal function and are typically increased in cases of advanced renal injury (Hojs *et al*., 2006). In the current study, this functional disorder was followed by structural alterations, including shrunken glomeruli, tubular dilation, congestion, inflammatory cell infiltration, necrosis, and interstitial edema. Similar histopathological observations were suggested by previous studies (Hojs *et al*., 2006; Fathi *et al*., 2021). As a result, we can conclude that EtOH is toxic to the kidney and leads to renal dysfunction. The ability of EtOH to impair renal function may be due to causing tubular injury and/or decreasing the glomerular filtration rate (GFR), resulting in the detention of nitrogenous waste such as urea, creatinine, and other products that are usually removed by the kidneys; therefore, their serum concentrations are increased (Lucas *et al*., 2019; Samadi *et al*., 2018).

In agreement with Jedidi *et al*. (2022) the administration of EtOH in our study led to a significant elevation in the production of H_2O_2, NO, and MDA in renal tissue, suggesting severe oxidative stress and lipid peroxidation. Previous experimental studies have revealed that EtOH can promote oxidative stress-dependent renal toxicity by inducing endoplasmic reticulum stress and stimulating the production of free radicals, such as superoxide radicals, hydroxyethyl radicals, peroxyl radicals (ROO$^{\bullet}$), hydroxyl radicals (OH$^{\bullet}$), and hydrogen peroxide (H_2O_2), from mitochondria (Samadi *et al*., 2018; Żukowski *et al*., 2018). As a result, increased ROS generation by EtOH leads to lipid peroxidation in the kidney (do Vale *et al*., 2017). Lipid peroxidation is a series of events that occurs when membrane lipids react with reactive oxygen or nitrogen species (RNS). This response may change the nature of the membrane, causing structural modifications that contribute to renal impairment (Yaribeygi *et al*., 2018). Under the same conditions, our findings showed a decline in the activities of the renal antioxidant system after treatment with EtOH,

which is consistent with previous research (Dogan and Anuk, 2019). In renal tissues, as in other tissues, there are enzymatic and nonenzymatic agents with antioxidant potential, such as SOD, CAT, and GPx, which are responsible for balancing several oxidative pathways and regulating the release of ROS inside the cells. Therefore, oxidative stress is initiated when the generation of ROS overrides the activities of the antioxidant system, inducing the destruction of several cellular macromolecules, such as lipids, proteins, and DNA (Gazia and El-Magd, 2019). Therefore, an imbalance between the antioxidant system and ROS generation in the current study could trigger renal oxidative injury and nephropathy. Both high cellular generation of ROS and the failure of endogenous defense status led to the production of secondary reactive species that in turn caused renal tissue damage and induced kidney function impairment. Therefore, excessive alcohol intake results in major tension in the normal metabolic mechanisms of the kidneys, causing prospective renal damage and hemodynamic deficiency (Leal et al., 2017).

On the other hand, Liu et al. (2015) have reported that EtOH could induce oxidative stress-induced apoptotic cell death by upregulating proapoptotic and downregulating antiapoptotic protein expression. This confirmed that the presence of pyknotic nuclei in most epithelium lining the tubules in our study was an indicator for the onset of apoptosis. Furthermore, EtOH consumption decreased the production of prostaglandin E2 in renal tissue, which can activate the secretion of anti-inflammatory cytokines and dilate blood vessels to raise blood flow in the glomeruli, inducing an immunoreaction response and abnormal glomerular hemodynamics, thereby causing harmful consequences on renal morphology and function (Elkomy et al., 2018). These processes may explain the occurrence of inflammatory cellular infiltration in the current study (EtOH-treated group). According to several studies on experimental animals, the unusual immunoreaction after ethanol intake could be due to acetaldehyde, the first metabolite of ethanol, which is able to form covalent adducts with various proteins to stimulate the immune response (Kaartinen et al., 2009). On the other hand, NO is a free molecule that is produced by epithelial cells of the renal tubule, and it has a crucial effect on glomerular and medullar hemodynamic balance. Under long-term EtOH consumption, NO binds to ROS to generate $ONOO^-$, which is a strong oxidizing substance that reacts with macromolecules in the renal tissue, inducing lipid and protein peroxidation (Sönmez et al., 2012; Hosseini et al., 2017).

Our study also illustrated that the findings of histopathological observations and biochemical examinations were consistent with each other, where histopathological evaluations in EtOH-treated rats with Masson's trichrome staining techniques illustrated that renal fibrosis noticeably increased compared to the control and other groups. Our results agreed with previous in vivo and in vitro research. These studies confirmed that alcohol consumption caused significant upregulation of fibrotic reactions in epithelial cells of renal tubules because of its ability to activate oxidative stress-induced DNA methylation (Yang et al., 2020) as well as upregulation of TGF-β mediators in kidney tissue. TGF-β is the main agent in renal fibrosis in both humans and experimental animals under renal pathological conditions through its ability to promote the production of extracellular proteins in addition to its effective role in the transformation of tubule epithelial cells into myofibroblasts (Budi et al., 2021).

Since ROS overproduction is the main process whereby ethanol causes structural and functional alterations in the kidney, drugs with antioxidant activities were qualified to provide prevention against the oxidative injury induced by ethanol in the renal tissue, thereby enhancing the structure and function of the kidney (do Vale et al., 2017). In the current work, cotreatment with AME led to a significant decline in the levels of serum creatinine and urea but not the level of uric acid and exhibited a remarkable reduction in histopathological alterations and oxidative stress markers, including H_2O_2, NO, and MDA, in renal tissue, along with enhancement in the activity of intracellular antioxidant parameters. These findings reflect the protective role of AME against the toxicity of EtOH. Therefore, it can be a perfect antioxidant and ROS scavenger. Our findings are in agreement with other research that proved the cytoprotective effect of myrrh via free radical removal and stimulation of antioxidant activities in various organs, such as the heart and stomach (Abdel-Daim et al., 2015; Lebda et al., 2021). The high level of uric acid after cotreatment with AME in the current study could reflect its effective role in the attenuation of oxidative stress. It has been suggested that uric acid might be one of the most significant antioxidants in mammalian body fluids (Nälsén et al., 2006). Nery et al. (2015) suggested that uric acid has a strong scavenging activity of free radicals such as hydroxyl radicals and peroxyl radicals, i.e., it exerts a useful effect in tissue repair. According to our previous research, we found by using GC-MS that sesquiterpenes are the most abundant contents in myrrh extract, especially the furano-sesquiterpenes class, including curzerene, aromadendrene 2, gamma-elemene, and β-elemene (Alahmari et al., 2022). Furano-sesquiterpenes are a particular class of sesquiterpenes that contain 15 carbons, one of which is furan-based (Dolara et al., 2000). Experimental studies on animals have

reported that furano-sesquiterpenes have a broad range of cytoprotective effects against various chemical or physical stimuli that induce cellular injury, where sesquiterpenes act as electron donors to break off the radical system via reaction with reactive oxygen species and then transform them into the most stable product (Tursunova *et al.*, 2021; Yousefi-Manesh *et al.*, 2021). Interestingly, this property contributed to the ability of AME to stimulate the biosynthesis of metabolites with thiol groups, which are known to protect cells from oxidative stress (Bone, 2003). Moreover, furano-sesquiterpenes not only serve as scavengers of ROS but also cause the production of endogenous antioxidants, which in turn are able to remove ROS (Younis and Mohamed, 2021). This may explain the improvement in TAC and antioxidant parameters, including SOD, CAT, GSH, and GPx, in rats treated with AME in the current study. On the other hand, the activity of most of the furano-sesquiterpenes, especially curzerene, might be due to their highly lipophilic properties, which allows them to absorb rapidly and easily distribute to tissues after oral administration of AME (Alqahtani *et al.*, 2021). The scavenging activity of AME against ROS could also be due to the presence of various effective constituents, rather than sesquiterpenes, including phenolics and flavonoids, as reported by Shalabi and Otaif (2022), which may have some therapeutic impacts on nephrotoxicity caused by EtOH. Gallic acid, which is the main phenolic compound in myrrh, is known to be an antioxidant and anti-inflammatory substance. Its chemical structure has three hydroxyl groups that are responsible for its capacity to scavenge free radicals (Giftson *et al.*, 2010). Consistent with our suggestion, previous literature demonstrated that gallic acid ameliorated oxidative injury caused by lindane in the hepatic and renal tissues of rats (Padma *et al.*, 2011). On the other hand, several studies have reported the effective action of gallic acid, which is obtained from different medicinal plants, against the toxicity of various soft organs. Karampour *et al.* (2019) have provided evidence that small molecular gallic acid-derived ingredients participate in regulating prostaglandin E2 (PGE2) and NO release, thereby promoting the activity of antioxidant enzymes and reducing inflammation by preventing immune cell infiltration in rat gastric ulcers caused by ethanol. Therefore, the role of myrrh in the attenuation of inflammation in our study might be one of the renal protective mechanisms. Our study also hypothesized that the renoprotective action of myrrh could occur through stimulation of the Nrf2 signaling pathway. The Nrf2 signaling pathway protects cells from oxidative injury by stimulating the intracellular antioxidant system (Badr *et al.*, 2019). Mahmoud *et al.* (2018) stated that phenolic contents in myrrh extract led to the upregulation of Nrf2 signaling in renal tissue, protecting the kidney from methotrexate.

CONCLUSION

This study concluded that cotreatment with AME improved renal damage in nephrotoxicity caused by EtOH in rats. This was achieved by attenuating oxidative stress, inflammation and kidney dysfunction, all of which were obvious in restored biochemical biomarkers and decreased renal injury scores as a result of its rich chemical content of furano-sesquiterpenes, which possess powerful antioxidant activities. However, further experimental studies are needed to explore the exact molecular pathways by which AME exerts this therapeutic effect and to determine whether AME is clinically applicable.

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