Potential role of angiotensin converting enzyme/neprilysin pathway and protective effects of omapatrilat for paracetamol-induced acute liver injury

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Abstract. The renin-angiotensin-aldosterone system (RAAS) is an important pathway that contributes to the pathophysiology of acute liver injury due to paracetamol toxicity. Omapatrilat, a RAAS-acting agent, inhibits both angiotensin converting enzyme (ACE) and neprilysin/neutral endopeptidase (NEP). The aim of the present study was to investigate the hepatoprotective effects of omapatrilat and examine the role of ACE/NEP pathway on the physiopathology of paracetamol toxicity. A total of 56 BALB/c mice were separated into seven groups: Control, 40 mg/kg omapatrilat only, 400 mg/kg paracetamol only, paracetamol and 140 mg/kg N-acetylcysteine and three groups with paracetamol and 10-40 mg/kg omapatrilat. Blood and liver tissue samples were studied through histopathological imaging, alanine transaminase (ALT) and aspartate transaminase (AST) liver function tests and oxidant/antioxidant biomarker measurements including superoxide dismutase (SOD), glutathione (GSH) and malondialdehyde (MDA). ACE and NEP activities were also measured. Histopathological analysis revealed that paracetamol toxicity resulted in a number of apoptotic and necrotic cells in liver tissue samples. By contrast, with 40 mg/kg omapatrilat administration in toxicity-induced mice, hepatocytes were significantly improved and exhibited similar appearance to the control group. Biochemical measurements also supported these histopathological results. Omapatrilat pretreatment provided a dose-dependent reduction in oxidative stress and reversed paracetamol toxicity indications by reducing ALT and AST activities, increasing SOD activity and GSH levels and reducing MDA levels. Dose-dependent increase of ACE and NEP enzymes in omapatrilat groups was also observed. The results demonstrated promotion of antioxidant activity by omapatrilat and suppression of oxidative stress associated with acute liver injury. These findings revealed the potential role of ACE/NEP pathway in paracetamol toxicity and hepatoprotective effects of omapatrilat against oxidative stress.

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Introduction

Paracetamol toxicity is one of the most common causes of drug-induced hepatotoxicity (1) and acute liver failure (2) due to the worldwide availability of paracetamol, its affordability and widespread use for the treatment of pain and fever (3). A number of studies are underway to clarify the pathophysiology of paracetamol toxicity and its metabolization and detoxification. Paracetamol is mainly metabolized in the liver through conjugation with glucuronic acid and sulfates and is eliminated through the urinary system (4). A small amount of paracetamol is metabolized by cytochrome P450 isoenzymes, creating the extremely toxic metabolite N-acetyl-p-benzoquinone imine (NAPQI). This metabolite significantly depletes glutathione (GSH) reserves (5,6) and binds covalently to hepatocyte membrane proteins. The standard treatment against this mechanism is N-Acetylcysteine (NAC), which is a precursor influencing GSH formation and preventing acute liver injury resulting from NAPQI. However, details of this underlying toxicity mechanism remain to be elucidated (7).

The renin-angiotensin-aldosterone system (RAAS) is one of the pathways that serves a role in the pathophysiology of acute liver injury due paracetamol toxicity. Some studies have shown that drugs influencing RAAS have potential preventive effects against paracetamol-induced hepatotoxicity (8,9). RAAS is a multi-hormonal system that is known to regulate systemic circulatory homoeostasis, but also contributes to pathophysiological mechanisms including inflammation and oxidative stress (10) as well as the pathogenesis of liver fibrosis (11-13). Angiotensin II (Ang II), a key mediator in

RAAS, releases free radical precursor enzymes such as nicotinamide adenine dinucleotide phosphate oxidase and xanthine oxidase into vascular structures and leads to increased free radicals (14,15). In the gastrointestinal system, increased Ang II is shown to promote hepatic inflammation and fibrosis during chronic liver disease (16). In addition, reduction in Ang II due to inhibition of angiotensin-converting enzyme (ACE) prevents oxidative stress and tissue damage (12,17). For instance, captopril, a well-known ACE inhibitor, is effective against paracetamol-induced hepatotoxicity (18). Similarly, aliskiren, which directly inhibits renin by downregulating RAAS, is also shown to promote antioxidant activity against paracetamol toxicity (19). As such, inhibition of ACE may potentially prove effective against paracetamol toxicity.

In addition to ACE, the vasoconstrictive response due to RAAS can also be modulated by neprilysin/neutral endopeptidase (NEP), an enzyme that degrades natriuretic peptides (20). Of these peptides, the concentration of atrial natriuretic peptide (ANP) increases due to NEP inhibition (21). ANP also prevents liver damage and mediates hepatoprotective action (22), as well as promoting antioxidant activity by enhancing the resistance of hepatocytes against reactive oxygen species (23). ANP is also known to inhibit the RAAS pathway (24). Consequently, an agent able to inhibit both ACE and NEP could provide even stronger hepatoprotection, which has not been studied or experimentally demonstrated previously, to the best of the authors' knowledge.

Omapatrilat, originally proposed as an antihypertensive agent (25), is a recently developed drug with effects on the RAAS pathway. Studies so far have shown that omapatrilat can prevent endothelial dysfunction (26), provide cardiovascular and kidney protection and reduce fibrosis (27,28). In contrast to the aforementioned drugs influencing RAAS, omapatrilat also inhibits NEP. The increase in natriuretic peptides due to this NEP inhibition and the decrease in Ang II by ACE inhibition both have beneficial effects in sepsis, as indicated by reductions in inflammation and oxidative stress (29-31). This combined inhibition of ACE and NEP suggests omapatrilat's hepatoprotective potential against paracetamol-induced toxicity. The present study aimed to investigate possible effects of omapatrilat's simultaneous ACE and NEP inhibition on hepatic pathology and study omapatrilat as a potential therapeutic target against paracetamol toxicity and acute liver injury for the first time, to the best of the authors' knowledge.

Materials and methods

Chemicals. Paracetamol, thiopental sodium, N-acetylcysteine and omapatrilat were purchased from Doğa Ilaç Hammaddeleri Tic. Ltd. Şti, IE. Ulagay Ilac Sanayii Turk A.S., Hüsnü Arsan İlaçları and Sigma-Aldrich (Merck KGaA), respectively.

Animals and drug regimen. A total of 56 BALB/c mice aged 14-15 weeks and weighing 30-35 g were housed in stainless steel cages under standard conditions (I12-h light/dark cycle; 21±2˚C and 55% relative humidity) and were given standard pellet feed and water *ad libitum*. All animal protocols were approved by Experimental Animal Ethics Committee of Ataturk University (approval no. 04.05.2018/108). The mice were fasted overnight, separated into seven groups (n=8) and administered the following chemicals according to the schedule depicted in Fig. 1.

Group 1: Control; Group 2 (OMA): Omapatrilat (40 mg/kg); Group 3 (PARA): Paracetamol (400 mg/kg); Group 4 (PARA + NAC): Paracetamol (400 mg/kg) + NAC (140 mg/kg, 2 doses); Group 5 (PARA + OMA10): Paracetamol (400 mg/kg) + omapatrilat (10 mg/kg), Group 6 (PARA + OMA20): Paracetamol (400 mg/kg) + omapatrilat (20 mg/kg); Group 7 (PARA + OMA40): Paracetamol (400 mg/kg) + omapatrilat (40 mg/kg).

All chemicals were administered orally by gastric gavage. Omapatrilat was administered at 10, 20 and 40 mg/kg doses, according to previous experimental studies which showed that omapatrilat can influence oxidative stress parameters at similar doses (31-33). For NAC, two doses were administered at 140 mg/kg each, as per its recommended therapeutic application against paracetamol poisoning (7,34). After 1 h following pretreatment (32,34,35) by omapatrilat or NAC, paracetamol was administered at 400 mg/kg, the standard dosage used in acute liver injury models (35-37). To avoid drug-food interactions, overnight fasting was applied (38,39). The mice were also fasted for 4 h after paracetamol administration to avoid possible interactions that could change drug bioavailability between groups. Finally, 24 h after paracetamol was administered, mice were given a 50 mg/kg dose of thiopental (intraperitoneal) anesthesia. Blood samples were collected into heparinized bottles by cardiac puncture, as is recommended for terminal stage of the study to collect a single, good quality and large volume of blood from the experimental animals. A total of ~1-2 ml of blood per mouse was collected by thoracotomy, which caused mortality. Livers were removed immediately after sacrifice.

Histopathological imaging. Liver tissue samples obtained from six mice per group were fixed in 10% formalin solution for 48 h at room temperature (22-24˚C), dehydrated using alcohols with increasing concentrations and cleared in xylene. In histopathological analyses, occasionally tissues can be lost during paraffinization and/or staining procedures. Multiple slides were made from each tissue, and histopathological damage was scored and analyzed after the best slides were selected. Samples were embedded in paraffin and sectioned to 5 μm slices using a Leica RM2235 microtome and disposable Leica 819 metal blades. The sections were stained with hematoxylin (5 min) and eosin (2 min) at room temperature (22-24˚C) and imaged using a light microscope. Images were then evaluated for the severity of tissue damage by an independent researcher who was blinded to the treatment groups, using the following scores: Apoptotic and necrotic cells in 5 different areas in each organ were counted and scored as 0 if apoptotic and necrotic cells were absent (0%), 1 for few (0-33%), 2 for moderate (33-66%) and 3 for more (66-100%). This score for each animal in each group was evaluated statistically based on the scores obtained from each slide. Necrotic cells are a form of cell swelling (oncosis) and burst due to loss of osmotic pressure. Apoptotic cells loose cell contacts and changes shape. Chromatin condenses in the nucleus and moves toward the nuclear envelope. Loss of water results in significant cell shrinkage and blebbing of the plasma membrane with little or

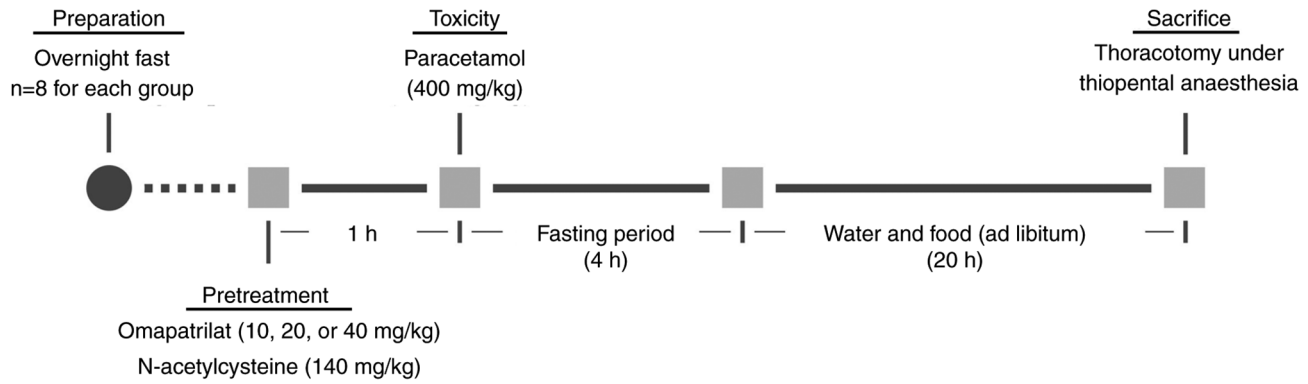

Figure 1. Schematic representation of drug regimen and scheduling.

no morphological changes to the other cellular organelles. The present study defined cells as apoptotic and necrotic according to these criteria, in the light of previous literature (7,38).

Biochemical measurements. In biochemical analyses there were seven samples for Control, eight samples for control + OMA and six samples for the rest of all PARA groups. The reason why there were different numbers of serum samples was loss of samples during collection (as a result of hemolysis). Serum samples were separated by a 10 min 1,800 x g centrifuge at 4˚C within 1 h of collection and were stored at -86˚C. For hepatic function assessment, alanine transaminase (ALT) and aspartate transaminase (AST) activities were characterized using Wuhan USCN Business Co., Ltd. ELISA kits (cat. nos. E90207Ra and E91214Ra) according to the manufacturer's instructions. Approximately 75 mg of ground liver tissue was homogenized in 1 ml of phosphate-buffered saline (PBS) using a homogenizer (TissueLyser II; Qiagen GmbH) and then centrifuged at (4˚C) 1,500 x g for 15 min. Total protein concentrations were measured using the Lowry method (total protein kit; cat. no. TP0300-1KT; MilliporeSigma). GSH levels were determined using an Mouse GSH ELISA kit (cat. no. E13068m; Cusabio Technology LLC) according to the manufacturer's instructions. Superoxide dismutase (SOD) activity (40) and malondialdehyde (MDA) levels (41) were measured manually from the supernatants, according to modified methods of the ELISA reader as previously described (42). Finally, levels of ACE (cat. no. E04492m; Cusabio Technology LLC) and NEP were measured (in ng/ml and pg/ml) using ELISA kits (cat. no. YLA1760MO; Shanghai YL Biotech Co., Ltd.) with a BioTek Epoch Microplate Spectrophotometer.

Statistical analyses. Results from biochemical measurements were analyzed using one-way analysis of variance (ANOVA) and Tukey's multiple comparison test using SPSS (version 20; IBM) and expressed as mean ± standard deviations. When the CONTROL group was compared with the other groups, $^{*}P<0.05$, $^{**}P<0.01$ and $^{***}P<0.001$ marks were used; when the PARA group was compared with the other groups, $^{\&}P<0.05$, $^{\&\&}P<0.01$ and $^{\&\&\&}P<0.001$ symbols were used and when the PARA + OMA groups are compared within themselves, $^{\#}P<0.05$, $^{\#\#}P<0.01$ and $^{\#\#\#}P<0.001$ marks were used.

Results from histopathological scoring were analyzed using Kruskal-Wallis followed by Dunn's test using SPSS (version 20; IBM), expressed as minimum to maximum. When the PARA group was compared with the other groups, $^{\&}P<0.05$, $^{\&\&}P<0.01$ and $^{\&\&\&}P<0.001$ symbols were used.

$P<0.05$ was considered to indicate a statistically significant difference.

Results

With omapatrilat administration in the OMA40 group (group 2), no adverse effects that would have prompted the discontinuation of this study were observed (such as >15% weight loss and/or the loss of the ability to walk or properly consume food/water).

Histopathological results. Samples of micrographs of the liver slices are shown in Fig. 2. There were no visible differences between the tissues extracted from mice that belonged to the same experimental group. All hepatic lobules in the Control group (group 1) presented normal size and morphology with healthy portal triads and central veins. No histopathological anomalies were observed for the hepatocytes and sinusoids in this group. Images from the OMA40 group (group 2) also showed similar portal triads, central veins, hepatocytes and sinusoids as the Control group, with no histopathological findings. By contrast, significant damage was observed in the lobules of the PARA group (group 3). A number of apoptotic cells with pyknotic nuclei and necrotic cells with abundant eosinophilic cytoplasm were recorded among the hepatocytes surrounding the portal vein. With the standard NAC treatment, a smaller number of apoptotic and necrotic cells were observed, despite some still remaining near the central vein. Compared to the PARA group, samples from the PARA + OMA10 group (10 mg/kg omapatrilat; group 5) showed fewer apoptotic and necrotic cells. This group exhibited sinusoidal dilatation and erythrocyte infiltration around the portal area. With 20 mg/kg omapatrilat (PARA + OMA20; group 6), significant differences from the PARA group were observed. Some eosinophilic cells were observed around the central vein, similar to the results from the PARA + NAC group (group 4). Finally, PARA + OMA40 group micrographs (40 mg/kg omapatrilat; group 7) showed no histopathological anomalies, with hepatocytes exhibiting almost identical appearance to those in the Control group. These assessments are summarized with comparative scores in Fig. 3 with relative indications of damage severity

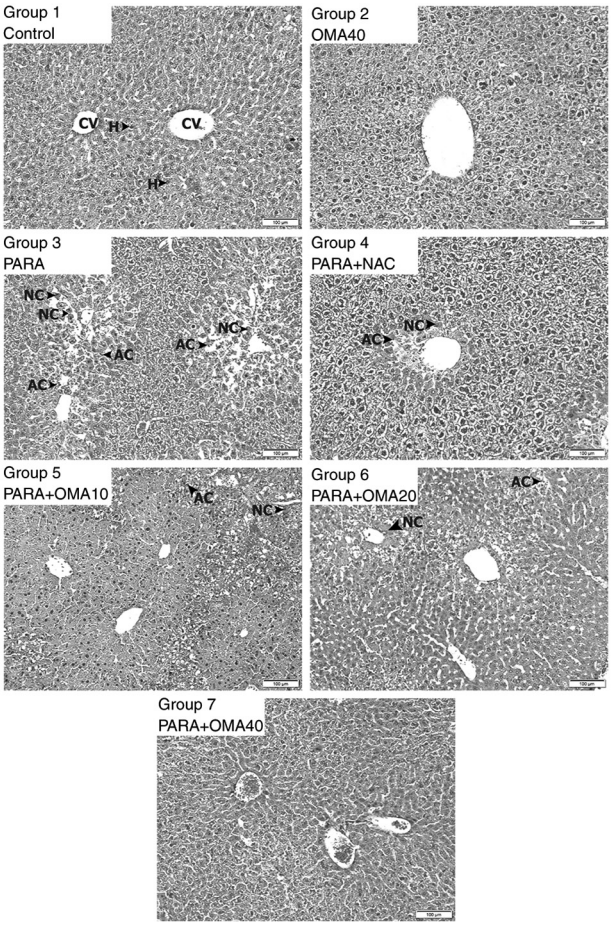

Figure 2. Micrographs of liver sections with hematoxylin & eosin stain in mice groups. Scale bar, 100 μm. Group 1: Control; Group 2 omapatrilat (40 mg/kg); Group 3 paracetamol (400 mg/kg); Group 4 paracetamol (400 mg/kg) + N-Acetylcysteine (140 mg/kg, 2 doses); Group 5 paracetamol (400 mg/kg) + omapatrilat (10 mg/kg), Group 6 paracetamol (400 mg/kg) + omapatrilat (20 mg/kg); Group 7 paracetamol (400 mg/kg) + omapatrilat (40 mg/kg). CV, central vein; H, hepatocytes; NC, necrotic cells; AC, apoptotic cells.

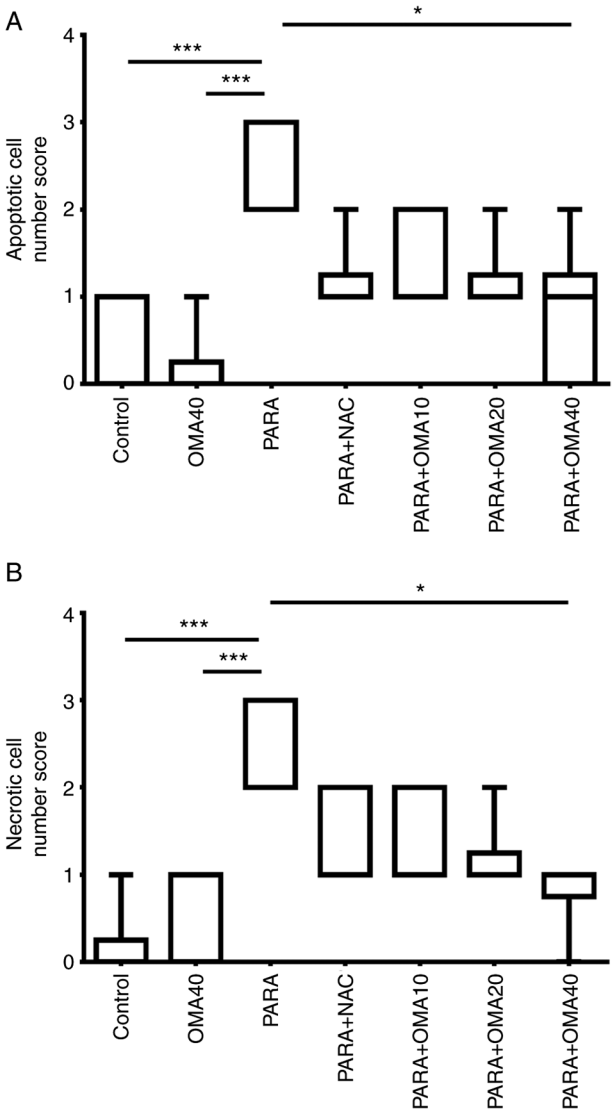

Figure 3. Histopathological scoring for comparative analysis of the damage resulting from paracetamol toxicity and omapatrilat treatment (n=6 for all groups). (A) Apoptotic cell number score, (B) necrotic cell number score from negative (0) to severe (3). Results from histopathological scoring were analyzed using Kruskal-Wallis followed by Dunn's test, expressed as minimum to maximum. *P<0.05 and ***P<0.001 PARA vs. other groups. For apoptotic cells NAC and OMA20 groups had decreased number of cells when compared to PARA group however these comparisons were borderline significant (P=0.0501 for PARA + NAC and PARA + OMA20 groups when compared to PARA according to Dunn's test). NAC, N-Acetylcysteine; PARA, paracetamol (400 mg/kg); OMA20, omapatrilat (20 mg/kg).

due to apoptotic and necrotic cells. From the histopathological scoring results it was determined that PARA group had the highest number of necrotic and apoptotic cells. A 40 mg/kg dose of OMA decreased these cell scores significantly. NAC and OMA20 groups has decreased number of apoptotic cells when compared to PARA group; however these comparisons were borderline significant (P=0.0501 for PARA + NAC and PARA + OMA20 groups when compared to PARA according to Dunn's test).

Biochemical results. According the liver function tests, significant increases in the serum activities of ALT and AST were observed in the PARA group compared with the Control and OMA40 groups, as shown in Fig. 4. The specific treatment was found to reduce ALT and AST activities in the PARA + NAC group, bringing them closer to those observed in the Control group. In groups pretreated with omapatrilat, it was seen that increasing omapatrilat dosage gradually reduced the ALT and AST activities, with the PARA + OMA40 group being the closest to the Control group. Comparing PARA + NAC and PARA + OMA administered groups, it was determined that 40 mg/kg dose of omapatrilat showed the closest results to the NAC-treated group. However, PARA + NAC administered group still had significantly lower AST and ALT levels than those in the PARA + OMA40 group.

As shown in Fig. 5, SOD activity and GSH levels in control and OMA40 groups were also statistically similar. Administration of paracetamol significantly reduced the SOD activity and the GSH level due to toxicity. This reduction was reversed with NAC pretreatment in the PARA + NAC group. Of the groups pretreated with omapatrilat, with increasing omapatrilat dosage from 10 to 40 mg/kg, it was seen that SOD activity and GSH level increased towards that measured in

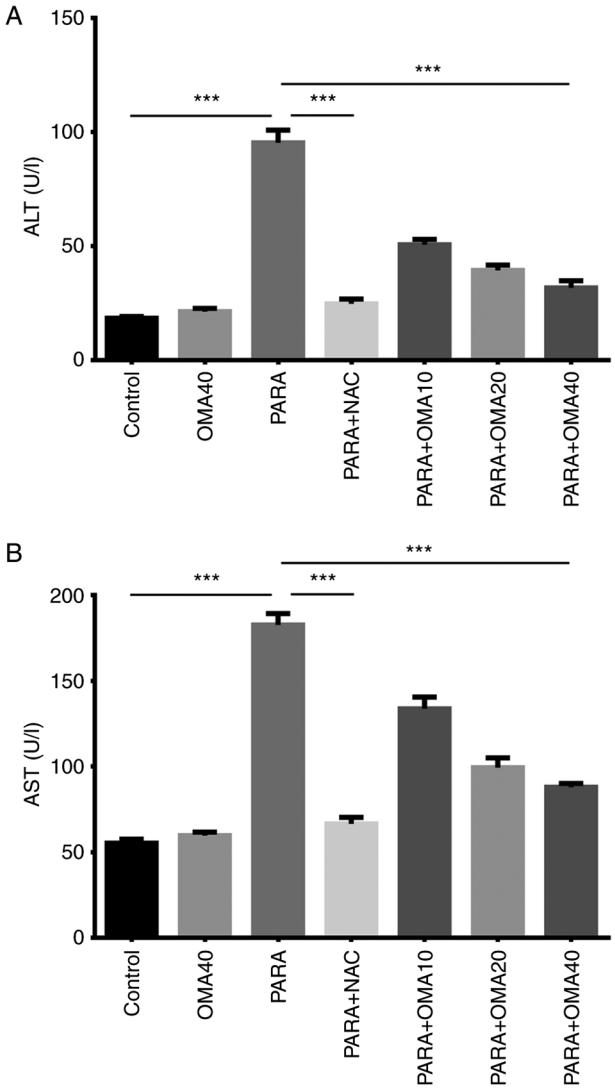

Figure 4. The effects of paracetamol toxicity and omapatrilat treatment on the serum activities of (A) ALT and (B) AST enzymes. Results are plotted as mean ± standard deviations (n=7 for Control, n=8 for control + OMA and n=6 for rest of all PARA groups). ***P<0.001 PARA vs. other groups. ALT, alanine transaminase; AST, aspartate transaminase; OMA, omapatrilat; PARA, paracetamol (400 mg/kg).

the control group, with the PARA + OMA40 group exhibiting the closest SOD and GSH levels to the control and OMA40 groups. Measurements of the oxidative stress biomarker MDA yielded similar results. Due to toxicity, introduction of paracetamol significantly increased the MDA level that was lower in the Control and OMA40 groups. Specific pretreatment in the PARA + NAC group also yielded results similar to the Control group. As with SOD and GSH, of the three groups pretreated with omapatrilat, PARA + OMA40 showed the closest results to the Control group, with increasing omapatrilat dosage gradually reversing the increase in the MDA level. Comparing the oxidative stress parameters and antioxidant status of PARA + NAC and PARA + OMA administered groups, it was determined that 40 mg/kg dose of omapatrilat was closest to the NAC-treated group. There was no significant difference between SOD and MDA levels in the PARA + NAC and PARA + OMA40 groups. Moreover, in comparison to the PARA group, the PARA + NAC group had increased GSH

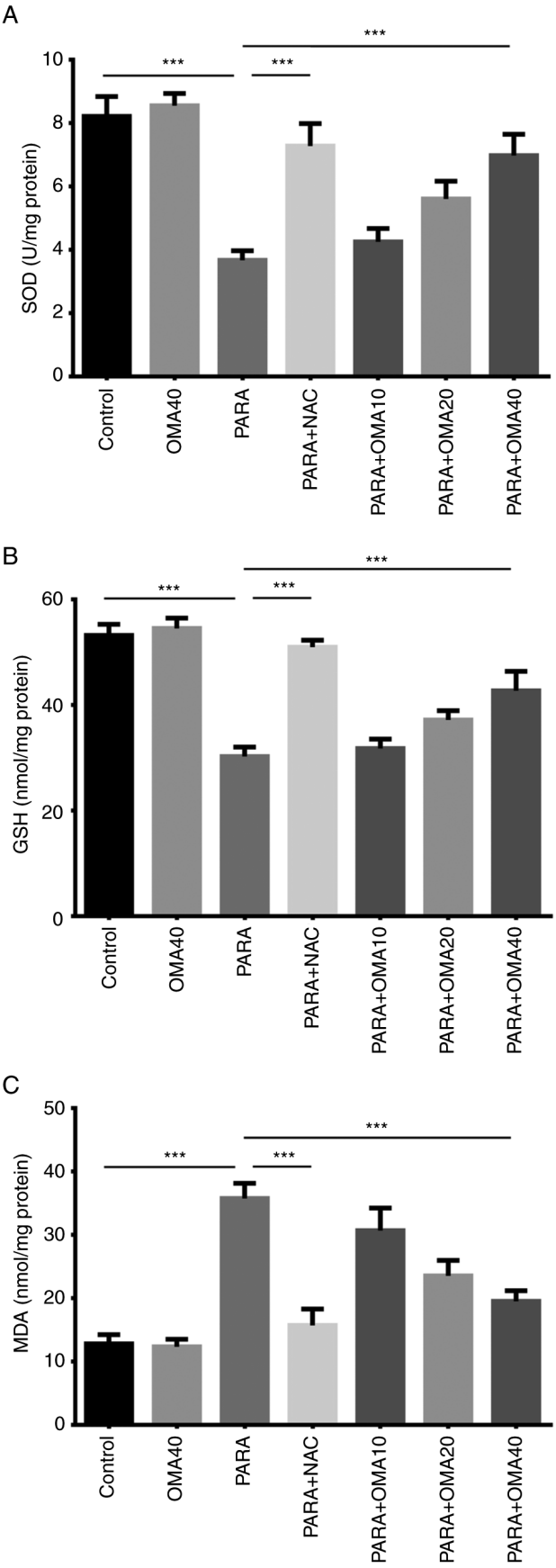

Figure 5. The effects of paracetamol toxicity and omapatrilat treatment on antioxidant/oxidant biomarkers (A) SOD, (B) GSH and (C) MDA. Results are plotted as mean ± standard deviations (n=7 for Control, n=8 for Control + OMA and n=6 for rest of all PARA groups). ***P<0.001 PARA vs. other groups. SOD, superoxide dismutase; GSH, glutathione; MDA, malondialdehyde; OMA, omapatrilat; PARA, paracetamol (400 mg/kg).

levels matching that of healthy mice. The next highest GSH levels were measured in the PARA + OMA40 group, below the control and PARA + NAC groups.

ACE and NEP levels shown in Fig. 6 were statistically higher in the PARA group than the Control and were both reduced by the standard treatment in the PARA + NAC group. In comparison to the control group, statistical increases in both ACE and NEP activities were observed with increasing omapatrilat dosage. All doses of omapatrilat resulted in significantly higher levels of ACE and NEP when compared with the PARA + NAC group.

Discussion

The present study investigated the protective effects of omapatrilat against paracetamol-induced hepatotoxicity. It was found that omapatrilat suppressed the acute liver damage caused by paracetamol, as evidenced by the histopathological results and the measurements of biochemical indicators. The histopathological results showed that in the case of specific treatment by NAC, no damaged cells were found, whereas the samples from the paracetamol toxicity group contained numerous apoptotic and necrotic cells. In groups pretreated with omapatrilat, histopathological results approached those of the Control group with increasing omapatrilat dosage. At 40 mg/kg of omapatrilat dosage, the tissue samples closely resembled the Control group and no histopathological findings were recorded. Also in the histopathological scoring results, the highest necrotic and apoptotic cell scores were determined in PARA groups. When PARA + NAC and PARA + OMA40 groups were evaluated, it demonstrated significant decrease in apoptotic and necrotic cell ratios. These findings were supported with biochemical analyses in which AST and ALT, serum biomarkers of liver function, were measured.

ALT and AST are enzymes synthesized in hepatocytes and are used in the evaluation of hepatocellular damage and are sensitive markers for the diagnosis of liver diseases. Previous studies have demonstrated significant changes in ALT and AST activities due to oxidative stress and liver damage resulting from the intake of toxic doses of paracetamol. For instance, paracetamol poisoning was shown to increase ALT and AST activities in rabbits (43). Another study on the effects of ACE inhibitor enalapril on paracetamol-induced liver damage has found significantly reduced ALT and AST activities in mice treated with enalapril (44). The present study investigated the effects of another ACE inhibitor, omapatrilat, on ALT and AST activities. The results also indicated that paracetamol administration results in much higher ALT and AST activities due to liver damage. The reduction of these enzymes with omapatrilat pretreatment towards those measured in the Control group is the primary evidence of omapatrilat's hepatoprotective activity.

Oxidative stress occurs because of increased free radical production, weakening antioxidant defense and a shift in the balance between oxidants and antioxidants in favor of oxidants. Oxidative stress and its biomarker MDA are also associated with paracetamol-induced liver damage (7). Paracetamol toxicity promotes reactive oxygen species production and initiates lipid peroxidation which causes tissue damage by affecting membrane structure and cell contents (45). As MDA is one of the end products of lipid peroxidation, elevated MDA

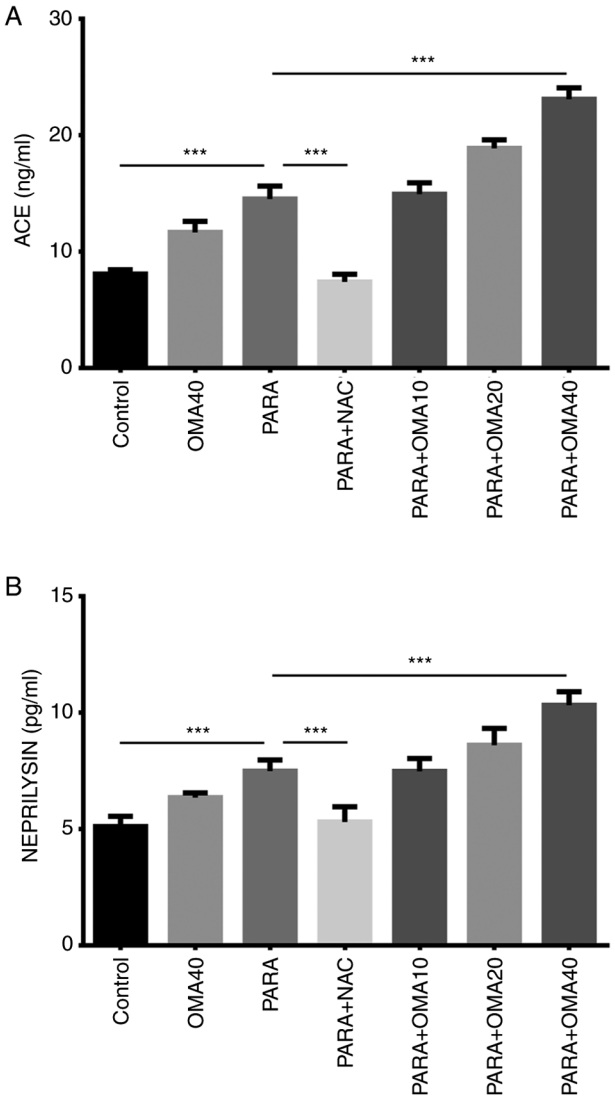

Figure 6. The effects of paracetamol toxicity and omapatrilat treatment on (A) ACE and (B) NEP activities. Results are plotted as mean ± standard deviations (n=7 for Control, n=8 for control + OMA and n=6 for rest of all PARA groups). Differences among experimental groups were considered to be significant ($P<0.05$). $^{***}P<0.001$ PARA vs. other groups. ACE, angiotensin converting enzyme; NEP, neprilysin/neutral endopeptidase; OMA, omapatrilat; PARA, paracetamol (400 mg/kg).

levels in the paracetamol toxicity group of the present study indicated oxidative stress-induced liver damage. As such, the reduction in the MDA levels for the omapatrilat groups demonstrated reduced oxidative stress and a strong hepatoprotective response by omapatrilat.

Glutathione (GSH), a crucial antioxidant, protects cells against oxidative damage by reacting with free radicals and peroxides (46). It is known that the amount of GSH significantly drops with paracetamol overdose and that N-acetylcysteine is the standard treatment for toxicity due to this glutathione depletion (47). This drop in GSH levels was also confirmed in the PARA group in the present study. Its measurements revealed that, with increasing omapatrilat dosage from 10 to 40 mg/kg, GSH content significantly increased. According to these results, it can be concluded that the increased amount of GSH prevented liver damage due to oxidative stress and renders the toxic metabolite NAPQI harmless.

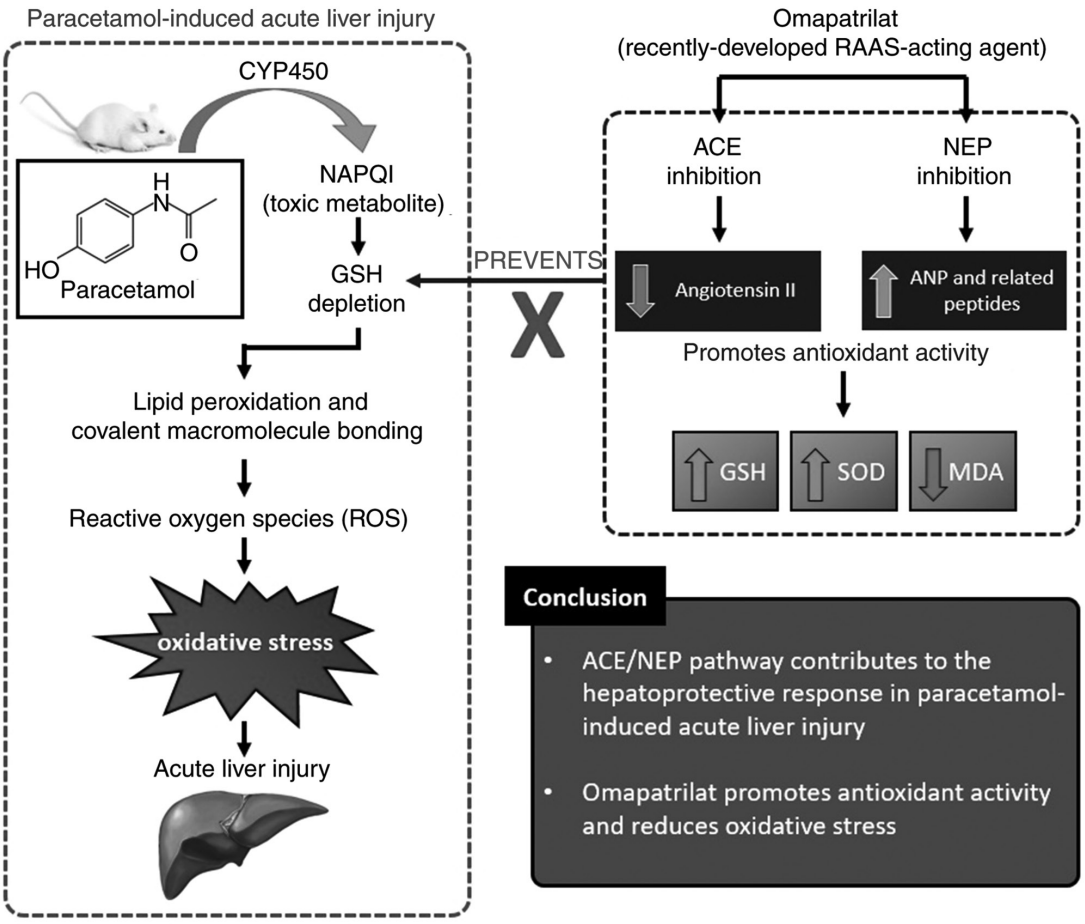

Figure 7. Graphical abstract of hypothesis and findings.

SOD measurements also confirmed these findings and proved the effectivity of omapatrilat against paracetamol toxicity. As SOD is an antioxidant enzyme, similar to the GSH measurements, SOD activity was also found to significantly decrease with paracetamol toxicity. As with GSH, increasing omapatrilat dosage also increased the SOD activity, with the PARA + OMA40 group approaching the Control group the closest. It has previously been shown that antioxidant activity in thiol-carrying antioxidants is directly influenced by the specific number of thiol groups and the oxidation state of sulfur atoms in the antioxidant molecule (48). Therefore, in addition to the antioxidant increase observed with the MDA, GSH and SOD results, the existence of a thiol group in omapatrilat may also contribute to the reduction of oxidative stress by promoting antioxidant activity.

Previous studies have found drugs effecting RAAS such as enalapril and aliskiren successful in increasing antioxidant levels and preventing liver damage due to oxidative stress (19,44). Of these drugs, omapatrilat, a more recently developed RAAS-acting agent, is the first one that is known to inhibit both ACE and NEP enzymes simultaneously. As such, the present study investigated the possible effects of ACE/NEP pathway on acute liver injury due to paracetamol toxicity and noted significant increases in the activities of both enzymes in the PARA group. This increase in ACE and NEP activities can be interpreted as a defense mechanism accompanying liver damage. Similar increases in ACE activity are also reported in case of acute pancreatitis in which the activation of local RAAS components in peripheral tissues are interpreted as differential mechanisms for regulation of physiological and pathophysiological functions (49). The potential pathophysiological role of increased ACE activity in acute coronary syndrome has also been previously shown (50). These earlier studies support our results that ACE activity can be influenced by acute phenomena. Moreover, the continued dose-dependent increase of ACE and NEP enzymes in omapatrilat groups may indicate deterioration of the negative feedback mechanism controlled by Ang II. These findings reveal the role of ACE and NEP enzymes and the preventive effects of omapatrilat in paracetamol toxicity.

There were limitations to the present study: i) Administration of omapatrilat before paracetamol demonstrated its protective effect, rather than its curative effect, on paracetamol-induced hepatotoxicity; ii) Lack of investigation on the potential combined effects between omapatrilat and NAC before and after paracetamol toxicity; and iii) the lack of molecular assays (immunofluorescence or western blotting) for the assessment of both ROS signaling and ACE-NEP pathway.

In summary, the results indicated that omapatrilat can be an effective hepatoprotective drug for paracetamol-induced hepatotoxicity. It showed that toxicity-induced mice pretreated with 40 mg/kg omapatrilat exhibited histopathological and biochemical results similar to the control group. It also showed that highest dose of omapatrilat (40 mg/kg)

resulted in similar protective effects as NAC. Omapatrilat, acting through the ACE/NEP pathway, demonstrated beneficial effects by correcting the antioxidant parameters and the GSH depletion resulting from paracetamol toxicity. Additionally, it was found that omapatrilat increased the antioxidant SOD activity and reduced the MDA oxidant levels by suppressing the oxidative stress associated with acute liver injury. Moreover, increase of ACE and NEP enzymes in omapatrilat groups may indicate deterioration of the negative feedback mechanism controlled by Ang II (Fig. 7). These measurements indicated the potential hepatoprotective effects of the ACE/NEP pathway on physiopathology of paracetamol toxicity. The aim of the present study our was not directly to suggest omapatrilat as a first aid agent after paracetamol toxicity, but to investigate possible contribution of ACE/NEP pathway during paracetamol toxicity. The present study cannot conclude that omapatrilat as an antidote but it shed light for future studies on omapatrilat and also other new agents targeting the ACE/NEP pathway.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

ZBAM, ZH and EC conceived the study and designed the experiments. ZBAM, ZH and RAU conducted the animal experiments under the supervision of EC. ET performed the histopathological imaging and analysis. ZBAM analyzed the data and wrote the manuscript with revisions from all authors. ZH and EC supervised the study. ZBAM, ET and RAU confirm the authenticity of all the raw data. All authors read and approved the final manuscript.

Ethics approval and consent to participate

All animal protocols were approved by Experimental Animal Ethics Committee of Ataturk University (approval no. 04.05.2018/108).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Penna A and Buchanan N: Paracetamol poisoning in children and hepatotoxicity. Br J Clin Pharmacol 32: 143-149, 1991.
2. Kurtovic J and Riordan S: Paracetamol-induced hepatotoxicity at recommended dosage. J Intern Med 253: 240-243, 2003.
3. Morthorst BR, Erlangsen A, Nordentoft M, Hawton K, Hoegberg LCG and Dalhoff KP: Availability of paracetamol sold over the counter in Europe: A descriptive cross-sectional international survey of pack size restriction. Basic Clin Pharmacol Toxicol 122: 643-649, 2018.
4. Steventon GB, Mitchell SC and Waring RH: Human metabolism of paracetamol (acetaminophen) at different dose levels. Drug Metabol Drug Interact 13: 111-117, 1996.
5. Jollow DJ, Mitchell JR, Potter WZ, Davis DC, Gillette JR and Brodie BB: Acetaminophen-induced hepatic necrosis. II. Role of covalent binding in vivo. J Pharmacol Exp Ther 187: 195-202, 1973.
6. Nilsson JLÅ, Blomgren A, Nilsson UJ, Högestätt ED and Grundemar L: N,N'-Bis(2-mercaptoethyl)isophthalamide binds electrophilic paracetamol metabolites and prevents paracetamol-induced liver toxicity. Basic Clin Pharmacol Toxicol 123: 589-593, 2018.
7. Yayla M, Halici Z, Unal B, Bayir Y, Akpinar E and Gocer F: Protective effect of Et-1 receptor antagonist bosentan on paracetamol induced acute liver toxicity in rats. Eur J Pharmacol 726: 87-95, 2014.
8. El-Demerdash E, Salam OM, El-Batran SA, Abdallah HM and Shaffie NM: Inhibition of the renin-angiotensin system attenuates the development of liver fibrosis and oxidative stress in rats. Clin Exp Pharmacol Physiol 35: 159-167, 2008.
9. Stokkeland K, Lageborn CT, Ekbom A, Höijer J, Bottai M, Stål P and Söderberg-Löfdal K: Statins and angiotensin-converting enzyme inhibitors are associated with reduced mortality and morbidity in chronic liver disease. Basic Clin Pharmacol Toxicol 122: 104-110, 2018.
10. Aroor AR, Demarco VG, Jia G, Sun Z, Nistala R, Meininger GA and Sowers JR: The role of tissue renin-angiotensin-aldosterone system in the development of endothelial dysfunction and arterial stiffness. Front Endocrinol (Lausanne) 4: 161, 2013.
11. Bataller R, Gäbele E, Schoonhoven R, Morris T, Lehnert M, Yang L, Brenner DA and Rippe RA: Prolonged infusion of angiotensin II into normal rats induces stellate cell activation and proinflammatory events in liver. Am J Physiol 285: G642-G651, 2003.
12. Bataller R, Gäbele E, Parsons CJ, Morris T, Yang L, Schoonhoven R, Brenner DA and Rippe RA: Systemic infusion of angiotensin II exacerbates liver fibrosis in bile duct-ligated rats. Hepatology 41: 1046-1055, 2005.
13. Friedman SL: Mechanisms of hepatic fibrogenesis. Gastroenterology 134: 1655-1669, 2008.
14. Mollnau H, Wendt M, Szöcs K, Lassègue B, Schulz E, Oelze M, Li H, Bodenschatz M, August M, Kleschyov AL, *et al*: Effects of angiotensin II infusion on the expression and function of NAD(P)H oxidase and components of nitric oxide/cGMP signaling. Circ Res 90: E58-E65, 2002.
15. Landmesser U, Spiekermann S, Preuss C, Sorrentino S, Fischer D, Manes C, Mueller M and Drexler H: Angiotensin II induces endothelial xanthine oxidase activation: Role for endothelial dysfunction in patients with coronary disease. Arterioscler Thromb Vasc Biol 27: 943-948, 2007.
16. Bataller R, Ginès P, Nicolás JM, Görbig MN, Garcia-Ramallo E, Gasull X, Bosch J, Arroyo V and Rodés J: Angiotensin II induces contraction and proliferation of human hepatic stellate cells. Gastroenterology 118: 1149-1156, 2000.
17. Hitomi H, Kiyomoto H and Nishiyama A: Angiotensin II and oxidative stress. Curr Opin Cardiol 22: 311-315, 2007.
18. Yeung JH: Effect of sulphydryl drugs on paracetamol-induced hepatotoxicity in mice. Drug Metabol Drug Interact 6: 295-301, 1988.
19. Karcioglu SS, Palabiyik SS, Bayir Y, Karakus E, Mercantepe T, Halici Z and Albayrak A: The role of RAAS inhibition by aliskiren on paracetamol-induced hepatotoxicity model in rats. J Cell Biochem 117: 638-646, 2016.

20. McDowell G, Coutie W, Shaw C, Buchanan KD, Struthers AD and Nicholls DP: The effect of the neutral endopeptidase inhibitor drug, candoxatril, on circulating levels of two of the most potent vasoactive peptides. Br J Clin Pharmacol 43: 329-332, 1997.
21. Mangiafico S, Costello-Boerrigter LC, Andersen IA, Cataliotti A and Burnett JC Jr: Neutral endopeptidase inhibition and the natriuretic peptide system: An evolving strategy in cardiovascular therapeutics. Eur Heart J 34: 886-893c, 2013.
22. Gerwig T, Meissner H, Bilzer M, Kiemer AK, Arnholdt H, Vollmar AM and Gerbes AL: Atrial natriuretic peptide preconditioning protects against hepatic preservation injury by attenuating necrotic and apoptotic cell death. J Hepatol 39: 341-348, 2003.
23. Kiemer AK, Gerbes AL, Bilzer M and Vollmar AM: The atrial natriuretic peptide and cGMP: Novel activators of the heat shock response in rat livers. Hepatology 35: 88-94, 2002.
24. Sekino M, Makita T, Ureshino H, Sungsam C and Sumikawa K: Synthetic atrial natriuretic peptide improves systemic and splanchnic circulation and has a lung-protective effect during endotoxemia in pigs. Anesth Analg 110: 141-147, 2010.
25. Kostis JB, Packer M, Black HR, Schmieder R, Henry D and Levy E: Omapatrilat and enalapril in patients with hypertension: The omapatrilat cardiovascular treatment vs enalapril (OCTAVE) trial. Am J Hypertens 17: 103-111, 2004.
26. Quaschning T, d'Uscio LV, Shaw S and Lüscher TF: Vasopeptidase inhibition exhibits endothelial protection in salt-induced hypertension. Hypertension 37: 1108-1113, 2001.
27. Dong Y, Zhou H, Shaffer E, Atamas N, Liao WC and Wei C: The cardiovascular actions of omapatrilat in spontaneously hypertensive rats. Curr Hypertens Rep 3 (Suppl 2): S1-S5, 2001.
28. Taal MW, Nenov VD, Wong W, Satyal SR, Sakharova O, Choi JH, Troy JL and Brenner BM: Vasopeptidase inhibition affords greater renoprotection than angiotensin-converting enzyme inhibition alone. J Am Soc Nephrol 12: 2051-2059, 2001.
29. Song Z, Cui Y, Ding MZ, Jin HX and Gao Y: Protective effects of recombinant human brain natriuretic peptide against LPS-Induced acute lung injury in dogs. Int Immunopharmacol 17: 508-512, 2013.
30. Kostakoglu U, Topcu A, Atak M, Tumkaya L, Mercantepe T and Uydu HA: The protective effects of angiotensin-converting enzyme inhibitor against cecal ligation and puncture-induced sepsis via oxidative stress and inflammation. Life Sci 241: 117051, 2020.
31. Ugan RA, Un H, Gurbuz MA, Kaya G, Kahramanlar A, Aksakalli-Magden ZB, Halici Z and Cadirci E: Possible contribution of the neprilysin/ACE pathway to sepsis in mice. Life Sci 258: 118177, 2020.
32. Hayek T, Hamoud S, Keidar S, Pavlotzky E, Coleman R, Aviram M and Kaplan M: Omapatrilat decreased macrophage oxidative status and atherosclerosis progression in atherosclerotic apolipoprotein E-deficient mice. J Cardiovasc Pharmacol 43: 140-147, 2004.
33. Lapointe N, Nguyen QT, Desjardins JF, Marcotte F, Pourdjabbar A, Moe G, Calderone A and Rouleau JL: Effects of pre-, peri-, and postmyocardial infarction treatment with omapatrilat in rats: Survival, arrhythmias, ventricular function, and remodeling. Am J Physiol Heart Circ Physiol 285: H398-H405, 2003.
34. Canayakin D, Bayir Y, Baygutalp NK, Karaoglan ES, Atmaca HT, Ozgeris FBK, Keles MS and Halici Z: Paracetamol-induced nephrotoxicity and oxidative stress in rats: The protective role of Nigella sativa. Pharm Biol 54: 2082-2091, 2016.
35. Randle LE, Sathish JG, Kitteringham NR, Macdonald I, Williams DP and Park BK: alpha(1)-Adrenoceptor antagonists prevent paracetamol-induced hepatotoxicity in mice. Br J Pharmacol 153: 820-830, 2008.
36. Patterson AD, Shah YM, Matsubara T, Krausz KW and Gonzalez FJ: Peroxisome proliferator-activated receptor alpha induction of uncoupling protein 2 protects against acetaminophen-induced liver toxicity. Hepatology 56: 281-290, 2012.
37. Scialis RJ, Ghanem CI and Manautou JE: The modulation of transcriptional expression and inhibition of multidrug resistance associated protein 4 (MRP4) by analgesics and their primary metabolites. Curr Res Toxicol 1: 34-41, 2020.
38. Palabiyik SS, Karakus E, Akpinar E, Halici Z, Bayir Y, Yayla M and Kose D: The role of urotensin receptors in the paracetamol-induced hepatotoxicity model in mice: Ameliorative potential of urotensin II antagonist. Basic Clin Pharmacol Toxicol 118: 150-159, 2016.
39. Ferah I, Halici Z, Bayir Y, Demirci E, Unal B and Cadirci E: The role of infliximab on paracetamol-induced hepatotoxicity in rats. Immunopharmacol Immunotoxicol 35: 373-381, 2013.
40. Sun Y, Oberley LW and Li Y: A simple method for clinical assay of superoxide dismutase. Clin Chem 34: 497-500, 1988.
41. Ohkawa H, Ohishi N and Yagi K: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95: 351-358, 1979.
42. Ugan RA, Cadirci E, Halici Z, Toktay E and Cinar I: The role of urotensin-II and its receptors in sepsis-induced lung injury under diabetic conditions. Eur J Pharmacol 818: 457-469, 2018.
43. Cigremis Y, Turel H, Adiguzel K, Akgoz M, Kart A, Karaman M and Ozen H: The effects of acute acetaminophen toxicity on hepatic mRNA expression of SOD, CAT, GSH-Px, and levels of peroxynitrite, nitric oxide, reduced glutathione, and malondialdehyde in rabbit. Mol Cell Biochem 323: 31-38, 2009.
44. Betto MRB, Lazarotto LF, Watanabe TT, Driemeier D, Leite CE and Campos MM: Effects of treatment with enalapril on hepatotoxicity induced by acetaminophen in mice. Naunyn Schmiedebergs Arch Pharmacol 385: 933-943, 2012.
45. Bessems JG and Vermeulen NP: Paracetamol (acetaminophen)-induced toxicity: Molecular and biochemical mechanisms, analogues and protective approaches. Crit Rev Toxicol 31: 55-138, 2001.
46. Karatas E, Bayraktutan Z and Cadirci E: Investigation of the effects of amlodipine on paracetamol-induced acute kidney toxicity in rats. Clin Exp Health Sci 12: 155-161, 2022.
47. Kalsi SS, Dargan PI, Waring WS and Wood DM: A review of the evidence concerning hepatic glutathione depletion and susceptibility to hepatotoxicity after paracetamol overdose. Open Access Emerg Med 3: 87-96, 2011.
48. Castañeda-Arriaga R, Pérez-González A and Galano A: Chemical protectors against the toxic effects of paracetamol (acetaminophen) and its meta analogue: Preventing protein arylation. ACS Omega 3: 18582-18591, 2018.
49. Ip SP, Kwan PC, Williams CH, Pang S, Hooper NM and Leung PS: Changes of angiotensin-converting enzyme activity in the pancreas of chronic hypoxia and acute pancreatitis. Int J Biochem Cell Biol 35: 944-954, 2003.
50. Hoshida S, Kato J, Nishino M, Egami Y, Takeda T, Kawabata M, Tanouchi J, Yamada Y and Kamada T: Increased angiotensin-converting enzyme activity in coronary artery specimens from patients with acute coronary syndrome. Circulation 103: 630-633, 2001.