Protective effect of *Celtis tournefortii* against copper-induced toxicity in rat liver

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Abstract

This study aimed to investigate the antioxidant and hepatoprotective effects of *Celtis tournefortii* fruit extract (Ct) against copper-induced liver damage in rats. Thirty-two Wistar Albino rats were divided into four equal groups (n = 8): Control, Copper (Cu), Copper + *C. tournefortii* (Cu+Ct), and *C. tournefortii* (Ct). Superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT) activities, glutathione (GSH) concentration, malondialdehyde (MDA), total antioxidant status (TAS) and total oxidant status (TOS) were analysed in the liver tissues. Liver histopathology was also evaluated. Alanine aminotransferase and lactate dehydrogenase significantly decreased in the Cu+Ct group compared with the Cu group. Oxidative stress parameters MDA and TOS significantly increased with copper administration, whereas they decreased with *C. tournefortii* co-treatment compared to Cu group. GSH concentration and TAS showed significant decreases with copper administration. *Celtis tournefortii* co-supplementation with copper significantly enhanced antioxidant parameters such as TAS, SOD, and GPx. *Celtis tournefortii* remarkably attenuated degenerative and necrotic changes caused by the oral exposure of copper in the liver tissue of rats. *Celtis tournefortii* may provide amelioration of the antioxidant status and moderation of severity of liver damage against copper toxicity. The therapeutic use of *C. tournefortii* may exhibit protective effects in liver injury treatment.

Antioxidant, hepatoprotective, oriental hackberry, phytochemicals, TAS, TOS

Copper (Cu) exposure can occur due to pesticide residue exposure (WHO 2003) in agriculture as well as via drinking water contaminated by environmental pollution or by copper water pipe corrosion (Brewer 2012). Excessive copper intake may also occur via consumption of Cu-rich foods such as liver, seafood, nuts, whole grains, and dried fruits. The tolerable upper intake level is 10 mg/day a value which represents the limit for protection of hepatic injury, a potentially critical side effect of excess copper ingestion (DRI 2001). Cu is a bioelement and a vital transition metal whose deficiency or excess in humans is associated with pathological situations, especially in liver and brain tissues. However, Cu metabolism is generally regulated by absorption and biliary excretion mechanisms. Cu chaperones, Cu transporters, and Cu-binding proteins/enzymes maintain physiological intracellular Cu homeostasis (Pal 2014). On the other hand, excess Cu causes production of free radicals (e.g. reactive oxygen species [ROS]) through Haber-Weiss and Fenton reactions, resulting in tissue damage (Valko et al. 2005). ROS-induced oxidative tissue damage plays an important role in Cu toxicity. For example, excess Cu may lead to peroxidative damage to membrane lipids via the reaction of lipid radicals and oxygen to form peroxy radicals, and causes peroxidation in the membranes of hepatocyte lysosomes. Also, Cu overload also reduces the activity of cytochrome c oxidase and impairs liver mitochondrial respiration (Gaetke et al. 2014).

Phytochemicals are bioactive compounds in plants and they exert a protective effect against various diseases (Zhang et al. 2015). Therefore, their nutraceutical use makes them

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important in terms of health. The antioxidant and hepatoprotective properties of plants have underscored the importance of plants in discovery of new medications with less side effects for use in hepatic disorder treatment. *Celtis tournefortii* fruit, commonly known as the "oriental hackberry", is palatable, nutrient-rich, and floury. *Celtis tournefortii* contains flavonoid and phenolic acids such as gallic acid, vanillic acid, ellagic acid, chlorogenic acid, ferulic acid, caffeic acid, rosmarinic acid, *p*-coumaric acid, myricetin, catechin, rutin, naringin, kaempferol, resveratrol, and quercetin (Keser at al. 2017; Yıldırım et al. 2017). *Celtis tournefortii* fruits are traditionally consumed for treatment of diarrhoea, dysentery, and peptic ulcers. Some *Celtis* species are used in epileptic seizures, foot perspiration, and as wound healing remedies. *Celtis tournefortii* seeds are used by the native population against kidney sand, whereas its leaves are used for stomach pain, menstrual bleeding, sedative purposes, and facilitating digestion (Keser et al. 2017). Although a few *in vitro* studies (Keser et al. 2017; Yıldırım et al. 2017) have investigated the antioxidant properties of this fruit, the protective effects against hepatotoxicity have not been investigated *in vivo*. This study exhibits an authentic value due to the acquisition of primary scientific data. Based on this assumption, we aimed to determine the antioxidant and hepatoprotective effects of *C. tournefortii* fruit extract (Ct) against copper-induced toxicity in rats.

Materials and Methods

Plant material

Celtis tournefortii fruits were collected from Siirt, Turkey, in August 2016. The plant was taxonomically identified and authenticated by Dr. Metin Armagan, Adnan Menderes University, Aydın. Fruits were dried under shade and powdered.

Celtis tournefortii fruit extraction

One g powdered *Celtis* fruit was added to a 100 ml beaker containing 50 ml solvent (water, ethanol 25%, and ethanol 75%, separately) and extracted by stirring for 3 h, at 50 °C and 750 rpm. The extract was then filtrated and centrifuged for 5 min at +4 °C, 3150 × *g* in a falcon tube. The obtained extract was freeze-dried for experimental study. Extraction was performed in duplicate for total phenolic and total flavonoid content analysis.

Determination of total phenolic and total flavonoid content

The total phenolic content (TPC) in the Ct extract was determined by modified Folin-Ciocalteau reagent method (Singleton and Rossi 1965) using gallic acid as a standard. The TPC was calculated as mg gallic acid equivalent for 100 g sample (mg GAE/100 g sample). The total flavonoid content (TFC) was determined by the $AlCI_3$ method (Zhishen et al. 1999) using quercetin as a standard. The TFC was calculated as mg quercetin equivalent for 100 g sample (mg QE/100 g sample). Analyses were triplicated.

Extract prepared with ethanol 25% was used in the animal study because of the highest amounts of TPC and TFC in this extract were determined.

Acute toxicity assay (LD50)

For determining whether *C. tournefortii* fruit extract has acute toxicity, an LD_{50} study was performed on rats for 7 days. Control and three plant extract groups (n = 6) were formed. The rats were fasted for 12 h before the plant extract was administered. The Ct was orally administered to rats with a bulbed steel needle at graded single doses (600, 1200, and 2400, $mg \cdot kg^{-1}$body weight [b.w.]), so a total amount of 24 animals were used. Rats were anaesthetized and sacrificed at the end of the toxicity assay. Then their bloods were collected for liver function test.

Animals and experimental protocol

Experiments on the effects of the Ct were performed on 32 Wistar Albino male rats (150-250 g and 2 months old) obtained from Experimental Application and Research Center, Yuzuncu Yil University (Turkey). Rats were kept at 22°C, 12:12 h light/dark cycle in separate stainless cages. They were fed *ad libitum* standard chow and tap water for 28 days. The experimental groups (n = 8) were randomly formed as follows:

Control group received 1 ml saline via gavage daily for 28 days.

Group 2 of rats (CS group) were administered 10 mg/kg b.w. copper sulphate via intragastric tube twice a week and received 1 ml saline via gavage on other days for 28 days based on the modified method by Temiz et al. (2018).

Group 3 of rats (CS + Ct group) were administered 10 mg/kg b.w. copper sulphate via intragastric tube twice a week and received 10 mg/kg b.w. Ct via gavage daily for 28 days.

Group 4 of rats (Ct group) received 10 mg/kg bw Ct via gavage daily for 28 days.

The present study was approved by Yuzuncu Yil University Animal Researches Local Ethics Committee (no. 27552122-604.01.02-E.7837) and procedures complied with the Guidelines for the Care and Use of Laboratory Animals.

Sample collection

Rats were anaesthetized with ketamine + xylazine at the completion of the experiment. Blood was taken from the heart of each rat by an injector and transferred to serum-separating tubes (SSTs). The tubes were centrifuged at 850 × *g* for 10 min for serum enzymes including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) which were analysed in an auto-analyser (Roche Modular PP, Roche Diagnostics, Mannheim, Germany). Serum copper levels were analysed with atomic absorption spectrometer (iCE 3000 Series, Thermo Fisher Scientific, MA, USA) ($y = 0.0857x + 0.0082$, $R^2 = 0.9997$). Liver tissues were dissected and rinsed by saline. Tissue samples for biochemical evaluation were stored at −80 °C until analysis.

Biochemical analyses

Liver tissues were homogenized in ice-cold phosphate buffered saline (PBS) (pH 7.4) using titanium probe homogenizer (Sonopuls HD 2200, Bandelin, Berlin, Germany) for 3 min and centrifuged at 8,570 × *g* for 30 min at +4 °C. The obtained supernatants of liver tissues of rats were used to analyse glutathione concentration (GSH) (Rizzi et al. 1988) and lipid peroxidation (malondialdehyde, MDA) by measuring thiobarbituric acid reactivity (Slater 1984), glutathione peroxidase (GPx) (Paglia and Valentine 1967), superoxide dismutase (SOD) (McCord and Fridovich 1969), and catalase (CAT) activity (Aebi 1984). Protein quantification was performed by modifying the method by Lowry. The total antioxidant status (TAS) and the total oxidant status (TOS) were performed using commercially available kit (Rel Assay Diagnostic, Turkey) as described by Erel (2004, 2005). The TAS method is based on antioxidants in the sample converting the $ABTS^{+}$ radical (2,2′-Azino-bis[3-ethylbenzothiazoline-6-sulfonic acid]) into the ABTS form (Erel 2004). The TOS method is based on conversion of ferrous (Fe^{+2}) ion complexes of the oxidants present in the sample to the ferric (Fe^{+3}) form by oxidation (Erel 2005). Oxidative stress index (OSI) is the ratio of TAS and TOS parameters used to express the status of oxidative stress in tissues. OSI was calculated as per the following formula:

OSI (arbitrary unit) = (TOS/TAS) × 100

Histopathology

Liver tissues were taken from the rats and fixed in ice-cold, freshly prepared 10% formaldehyde for 72 h. Routine paraffin embedding and staining with haematoxylin-eosin were then performed on the liver tissue. The stained sections were evaluated by imaging with a light microscope (80i-DS-Ri2, Nikon, Tokyo, Japan). Histopathological results were evaluated semi-quantitatively according to the degree of the lesion as (-): none; (+): mild; (++): moderate; (+++): severe. Z ratio test was performed for the importance of the difference between the groups (n = 8) (Minitab 14 statistical software package).

Statistical analysis

Data were expressed as mean ± standard deviation. Significant differences between groups were assessed using one-way analysis of variance followed by Tukey's test and Tamhane's T2 (SPSS 18 statistical software package). *P* values ≤ 0.05 were accepted as significant.

Results

The TPC and TFC results of *C. tournefortii* fruit extracts are presented in Fig. 1A-B (Plate IX). The highest amounts of TPC and TFC in the extract were determined in extract prepared with ethanol 25% (25% ethanol/75% water) compared with pure water and ethanol 75% (ethanol 75%/25% water). This extract with ethanol 25% was then chosen for the treatment of experimental animals.

In the lethal dose (LD_{50}) study, no Ct group exhibited mortality. There was no reduction in the physical activity of the rats, no change in their colour of the fur, and no molting. No unusual changes observed in eye coloration, urine colour (bloody urine, dark brown colour etc.), and stool of all treated rats. In the LD_{50} toxicity study, dose-dependent increases in AST, ALT, LDH, and ALP levels were not significantly seen (Table 1).

Liver serum enzyme levels were used as biochemical markers for early acute hepatic damage (Table 2). Oral exposure of rats to copper significantly increased liver serum enzyme levels compared with control ($P < 0.05$). However, significantly lower values

Table 1. Serum indicators of LD_{50} toxicity of rats.

Serum	CG	600 mg/kg	1 200 mg/kg	2 400 mg/kg
AST (U/l)	120.5 ± 15.6	126.0 ± 11.0	127.7 ± 28.5	144.5 ± 30.8
ALT (U/l)	35.7 ± 3.7	36.3 ± 3.1	37.8 ± 6.4	38.5 ± 5.2
LDH (U/l)	1061 ± 298	1155 ± 170	1292 ± 299	1401 ± 240
ALP (U/l)	263.7 ± 56	255.3 ± 19.1	279.3 ± 32.3	284.2 ± 32.6

CG - control group; AST - aspartate aminotransferase; ALT - alanine aminotransferase; LDH - lactate dehydrogenase; ALP - alkaline phosphatase.

Table 2. Serum indicators of rats.

Serum	CG	Cu	Cu+Ct	Ct
AST (U/l)	119.0 ± 3.9	153.4 ± 18.0[a]	132.5 ± 11.5[a]	112.9 ± 13.2[b]
ALT (U/l)	34.6 ± 2.4	41.0 ± 1.5[a]	35.9 ± 4.5[b]	34.5 ± 2.6[b]
LDH (U/l)	1445 ± 318	1783.5 ± 268[a]	1589 ± 145[b]	1227 ± 129[b]
ALP (U/l)	272.5 ± 26	295.0 ± 69.4[a]	273.4 ± 56.3	219.3 ± 27.1[b]
Cu (mg/l)	1.048 ± 0.076	1.491 ± 0.278[a]	0.965 ± 0.188[b]	0.475 ± 0.097[a,b]

CG - control group; Cu - copper group; Cu+Ct – copper + *Celtis* group; Ct - *Celtis* group; AST - aspartate aminotransferase; ALT - alanine aminotransferase; LDH - lactate dehydrogenase; ALP - alkaline phosphatase; Cu - copper.
[a] - significantly different from the control group ($P < 0.05$); [b] - different from the Cu group ($P < 0.05$).

of ALT and LDH were noted in the Ct co-treatment with copper when compared to the Cu group. The amount of serum copper decreased in the Cu+Ct group compared to the Cu group ($P < 0.05$).

The liver MDA content, TOS, and OSI as oxidative stress parameters were significantly increased after Cu administration to rats regarding the control group, indicating the liver ROS generations and oxidative stress induction. Conversely, Ct co-supplementation with copper significantly restored these indices (Plate X-XI, Fig. 2A-C). However, TOS increased in the Cu+Ct group in comparison with control, despite being significantly lower than in the Cu group ($P < 0.05$). While liver TAS decreased in the Cu group, it increased with Ct treatment (Plate XI, Fig. 2D). The TAS was significantly higher in the Ct group compared to all groups.

Subacute copper exposure to rats showed severe inhibitory response on the liver's antioxidant status (Plate XII-XIII, Fig. 3). Liver GSH content and SOD, GPx, and CAT activities were significantly decreased in Cu-exposed rats than in control group, indicating suppressed liver antioxidant defence against ROS. Ct co-treatment with copper significantly recuperated liver SOD and GPx activities compared to the Cu group. Treatment with only *C. tournefortii* showed significant increases of antioxidant enzyme activities and GSH than Cu+Ct group (Plate XII-XIII, Fig. 3A-D).

Results of histopathological changes and lesions for both control and treated rats are shown in Table 3. No histopathological findings were observed in microscopic examination of the liver tissue of the rats in the control group or with Ct alone. Hepatocytes and portal areas had normal appearance, and remark cord around vena centralis was found regularly. The sinuses between the remark cords were normal (Plate XIV, Fig. 4A,D). Conversely, disseminated centrilobular hepatocellular degeneration and vacuolar degeneration were observed in the liver of Cu-treated rats. Mononuclear cell infiltration was detected in portal areas where lymphocytes with inflammatory cells were predominant. Because of dilatation

in the sinusoid, dissociation was remarkable in the remark cord structure. The cytoplasm of some hepatocytes had eosinophilic, pyknotic, and karyolytic nuclei. Focal coagulation necrosis was detected, especially in periacinar regions, where partial proliferation in Kupffer cells was observed (Plate XIV, Fig. 4B). Mild degenerative changes were observed in the livers of rats treated with Ct and copper. However, lymphocytic mononuclear cell infiltrations were rarely seen (Plate XIV, Fig. 4C).

Table 3. Histopathological changes in the control, Cu, Cu+Ct, and Ct groups of rats.

Changes/lesions in liver	Control	Cu	Cu+Ct	Ct
1. Dilatation of sinusoid	-/8	8/8[a]	3/8	-/8[b]
slight	*	*	3	*
moderate	*	5	*	*
severe	*	3	*	*
2. Hepatocellular degeneration	-/8	8/8[a]	4/8[b]	-/8[b]
slight	*	*	2	*
moderate	*	5	1	*
severe	*	3	1	*
3. Coagulation necrosis	-/8	8/8[a]	4/8[b]	-/8[b]
slight	*	1	1	*
moderate	*	5	3	*
severe	*	2	*	*
4. Inflammation cell infiltration	-/8	7/8[a]	3/8	-/8[b]
slight	*	2	2	*
moderate	*	3	1	*
severe	*	3	*	*

Cu - copper group; Cu+Ct – copper + *Celtis* group; Ct - *Celtis* group.
* - none; [a] - significantly different from control ($P < 0.05$); [b] - significantly different from the Cu group ($P < 0.05$); [c] - significantly different from the Cu+Ct group ($P < 0.05$).

Discussion

Presently, the use of phytochemicals is preferred to synthetic supplements (vitamins, minerals, fibre etc.) due to their beneficial pharmacological effects, as they are considered safer and more reliable (Kioukia-Fougia et al. 2016; Scott et al. 2020). These effects could be attributed to the presence of valuable constituents (Zhang et al. 2015; Keser et al. 2017; Yıldırım et al. 2017). In this study, antioxidant and hepatoprotective effects of *C. tournefortii* fruit were investigated against hepatic damage caused by Cu-toxicity in rats. This study exhibits an authentic value due to the acquisition of primary scientific data.

As regards to the TPC and TFC results, the 25% hydroalcoholic solvent revealed considerable amount of polyphenolic compounds in the extract compared to pure water and 75% ethanol. Yıldırım et al. (2017) found similar results for TPC in the methanolic extract. However, TFC was higher in the present study than the results of Yıldırım et al. (2017). The time, temperature and solvent preference in the extraction process may have affected the amount of compounds in the TFC (Temiz and Temur 2017). In addition, the sampling site, the quality of soil, the season etc. may have probably had an effect.

High-dose Ct extract did not cause death in the LD_{50} study, nor did it alter any physical condition and activity on the rats. However, a non-significant dose-dependent increase in serum parameters was detected (Table 1). It has been estimated that oral LD_{50} value was > 2400 mg/kg b.w. Ntchapda et al. (2008) stated that the LD_{50} dose of *Celtis durandii*

leaf extract were 14.10 g/kg with a mortality rate of 42%. In accordance with the current study, the *Celtis durandii* leaf extract caused an increase in both ALT and AST serum levels (Ntchapda et al. 2008). Another study conducted with a *Celtis iguanaea (Jacq.) Sarg.* leaf extract considered LD_{50} to be higher than 2,000 mg/kg and lower than 5,000 mg/kg (Gonçalvez et al. 2015). Therefore, high doses of *C. tournefortii* fruit extract may have low toxicity.

Studies have indicated that various forms of copper such as ion, compound, micro and nanoparticle lead to liver damage and cause increased liver serum enzymes including AST, ALT, and ALP (Lee et al. 2016; Arafa et al. 2017; Khalid et al. 2018; Temiz et al. 2018). Transaminase enzyme levels rise in serum as biomarkers of hepatotoxicity when the liver cell integrity is disrupted and parenchymal cells degenerated. However, *Celtis* treatment of Cu-induced liver-damaged rats contributed to recovery. There is limited data on *Celtis* spp. use for liver injury. Reportedly, administration of 100 mg/kg aqueous ethanolic leaf extract of *Celtis integrifolia* exhibited significant reduction in serum biochemical indicators such ALT and ALP (Geidam and Adole 2014). Polyphenols may not only act as antioxidants terminating free radical chain reactions but also as effective chelators of redox-active metals (Jomova and Valko 2011). Studies on flavonoids such as quercetin, catechin, and rutin have been conducted for discrimination between its antioxidant versus metal ion chelating properties in the red blood cell haemolysate system *in vitro*. The results showed that flavonoids maintained their efficiency to chelate redox-active metals (Cherrak et al. 2016). Serum copper levels dramatically reduced with *C. tournefortii* treatment concomitantly with Cu. *Celtis* contains flavonoids which, like a chelator, may have assisted the reduction of copper concentration in the serum by increasing copper excretion. Flavonoids are capable to form stable metal complexes through their multiple OH groups and the carbonyl moiety. For instance, quercetin which is characterized by three potential bidentate binding sites (α-hydroxy-carbonyl, β-hydroxy-carbonyl or catechol), can lead to stable metallic complexes. Previous investigation demonstrated that flavonoids are able to complex Cu^{2+}. The Cu-quercetin complexation was suggested to occur via the 4-keto group of the C-ring with additional involvement of the 3OH or 5OH group (Cherrak et al. 2016).

In the current study, MDA as well as TOS and OSI, which are an indicator of oxidant/antioxidant imbalance, exhibited a significant increase in Cu-administered rats (Fig. 2). Besides, TAS is formed by internal and external antioxidant molecules of the cell which act synergistically. Therefore, measurement of the total antioxidant capacity gives more valuable information than separate measurements. Findings of this study exhibited that Ct co-treatment with Cu reduced oxidation and increased the antioxidant status compared to the Cu group. TAS was significantly higher in the Ct group in comparison with all groups (Fig. 2D). This result showed better the antioxidant effect of Ct administered alone. In previous studies, the ethanolic extract of *C. australis* and *C. occidentalis* leaves (El-Alfy et al. 2011) and the hydromethanolic extract of *C. australis* leaves (Filali-Ansari et al. 2015) significantly reduced *in vitro* MDA formation. The present findings were consistent with Zanchet et al. (2018) who reported that a *C. iguanaea* supplement significantly decreased lipid peroxidation in cholesterol-fed rats by increasing SOD activity. Moreover, Arafa et al. (2017) reported that in Cu-induced liver, the elevated hepatic ROS and suppressed total antioxidant capacity improved with quercetin treatment. *Celtis tornefortii* also contains quercetin and other flavonoids as well as other aforementioned phenolic antioxidants (Keser et al. 2017).The decrease in lipid peroxidation and TOS in the treatment with Ct may be due to the presence of scavenger compounds. Furthermore, animals co-treated with Ct showed a significant improvement in the GSH content, activities of SOD and GPx compared with Cu-intoxicated rats (Fig. 3). These improvements in antioxidant enzymes conform to previous studies and can be attributed to antioxidant properties of the *Celtis* species (Dasari et al. 2013; Fall et al. 2017).

The present and previous studies have demonstrated that Cu induces severe histological changes and hepatic damage (Li et al. 2008; Ibrahim et al. 2015). Li et al. (2008) stated that Cu can induce liver damage through an up-regulation of apoptosis regulator B-cell lymphoma-2 associated protein X (Bax) expression. Conversely, co-treatment with Ct ameliorated liver injury, showing less degeneration and necrotic changes. *Celtis* co-treatment with copper may prevent liver injury or lead to the recuperation of damaged liver parenchyma. Phenolic and flavonoid compounds in herbs may have beneficial hepatoprotective effects. Studies indicated that many phytochemicals such as quercetin against Pb (Liu et al. 2013), gallic acid against NaF (Nabavi et al. 2013), and rutin against Cd (Mirani et al. 2012) effectively prevented hepatic damage against metal intoxication. Mechanisms underlying the effects of these compounds may be attributed to inhibiting the Fenton-like reaction, inhibiting the formation of highly reactive hydroxyl radicals by acting as a radical scavenger donating hydrogen (Ibrahim et al. 2015). Moreover, phytochemicals can induce the expression of endogenous antioxidants, and prevent ROS-mediated oxidative stress on DNA by exerting their antioxidant and anti-apoptotic effects (Ibrahim et al. 2015). Therefore, these phytochemicals may be responsible for the hepatoprotective effects of the *Celtis* extract. Polyphenolic compounds may synergistically modulate fibrosis and necrosis in the liver. Herbal medicines are mixtures of various phytochemicals that exert synergistically their full beneficial effect in total extracts (Zhang et al. 2019).

In conclusion, the hepatotoxicity of Cu was corroborated by the data obtained with increasing oxidative stress markers and hepatic degeneration and necrotic changes. However, it was revealed that *C. tournefortii* may have hepatoprotective effects against Cu-induced liver damage due to mitigated oxidative stress indicators and hepatocellular degeneration as well as on an enhanced antioxidant status. The use of *C. tournefortii* as nutraceutical for the maintenance of oxidant/antioxidant balance for liver damage may be efficacious.

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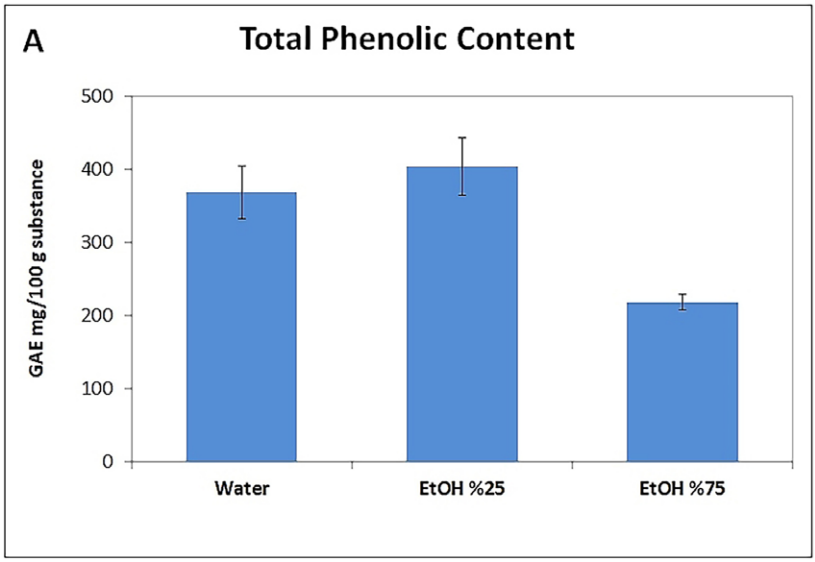


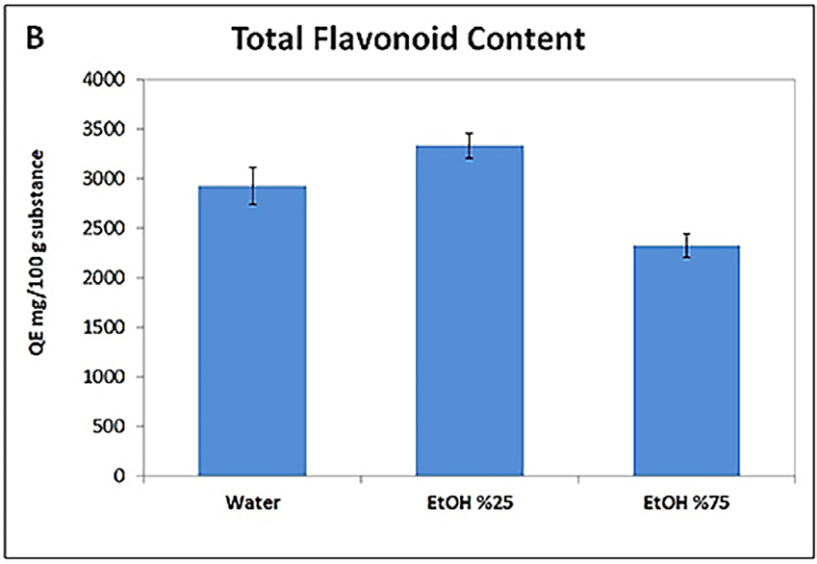
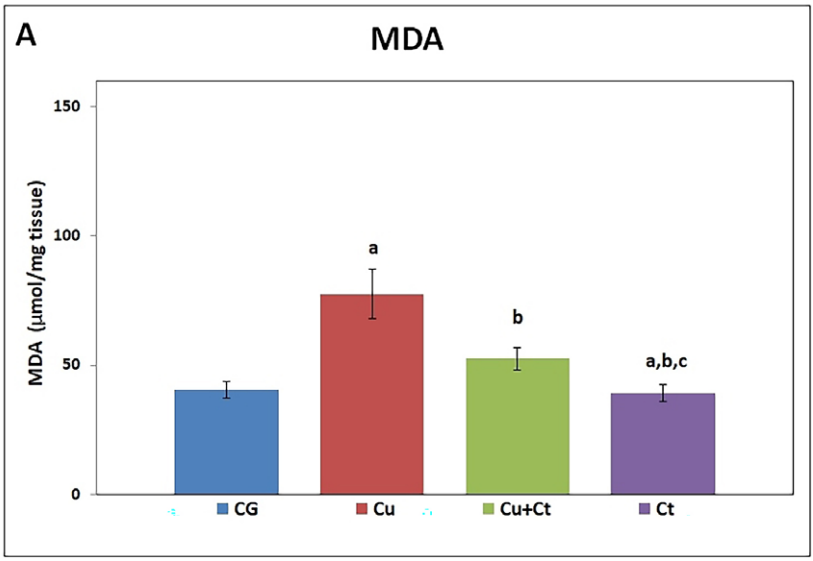
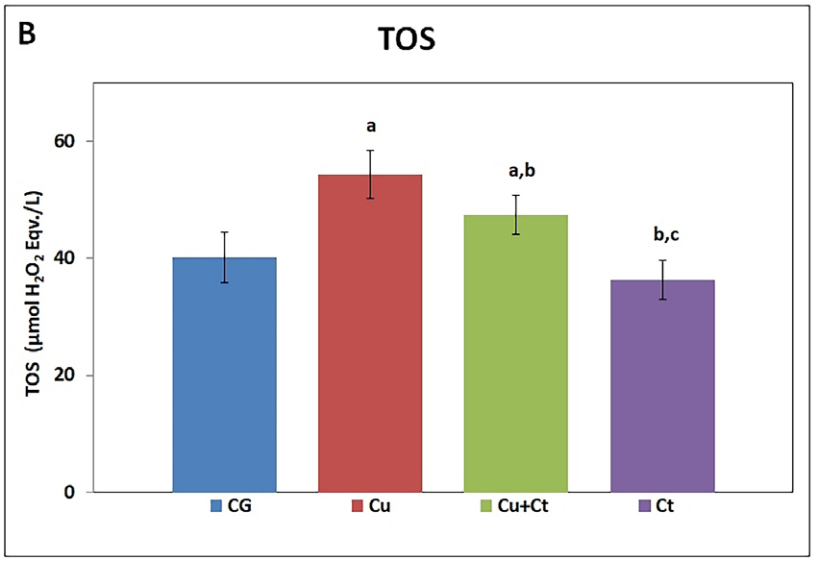
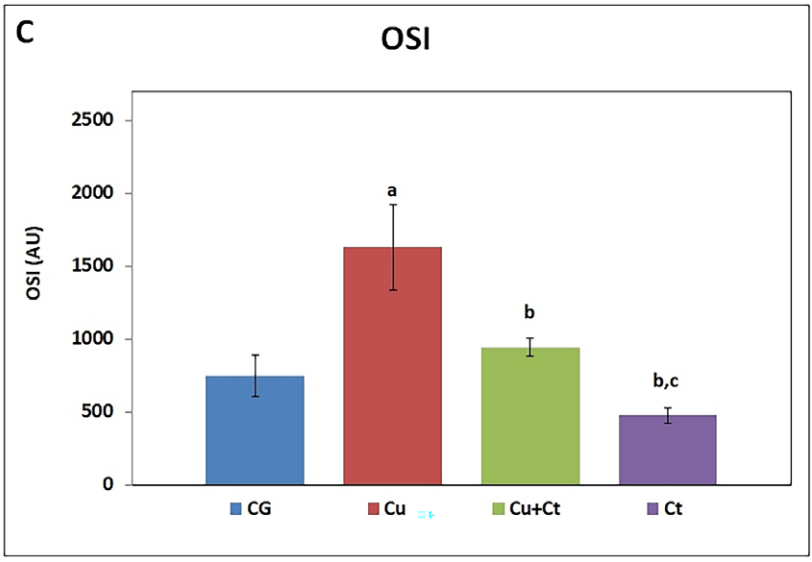


Fig. 1. The total phenolic content (A) and the total flavonoid content (B) in the *Celtis tournefortii* fruit extract.

Plate X


A
MDA
MDA (µmol/mg tissue)
150
100
50
0
a
b
a,b,c
CG
Cu
Cu+Ct
Ct


B
TOS
TOS (µmol H_2O_2 Eqv./L)
60
40
20
0
a
a,b
b,c
CG
Cu
Cu+Ct
Ct



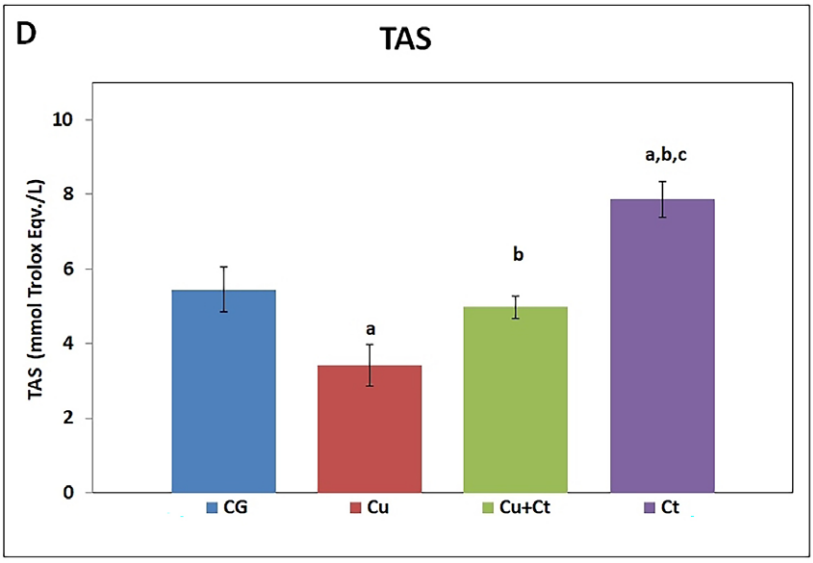
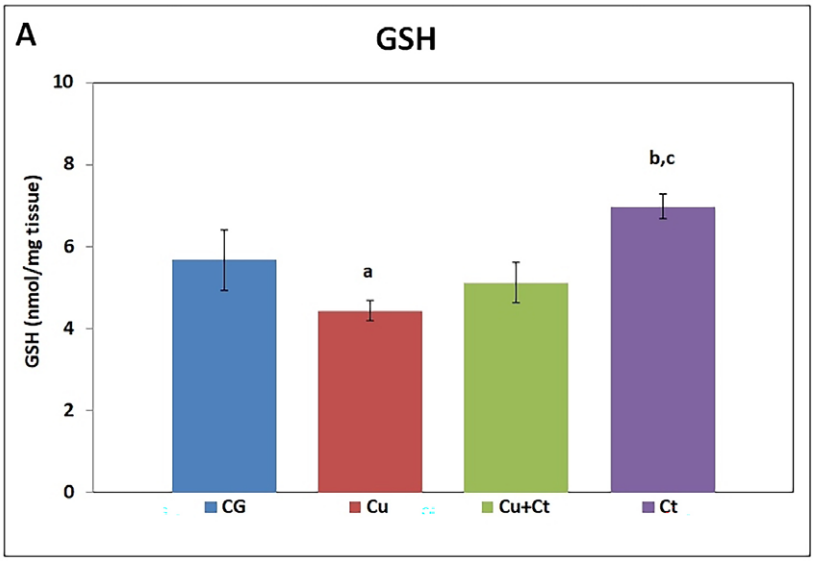
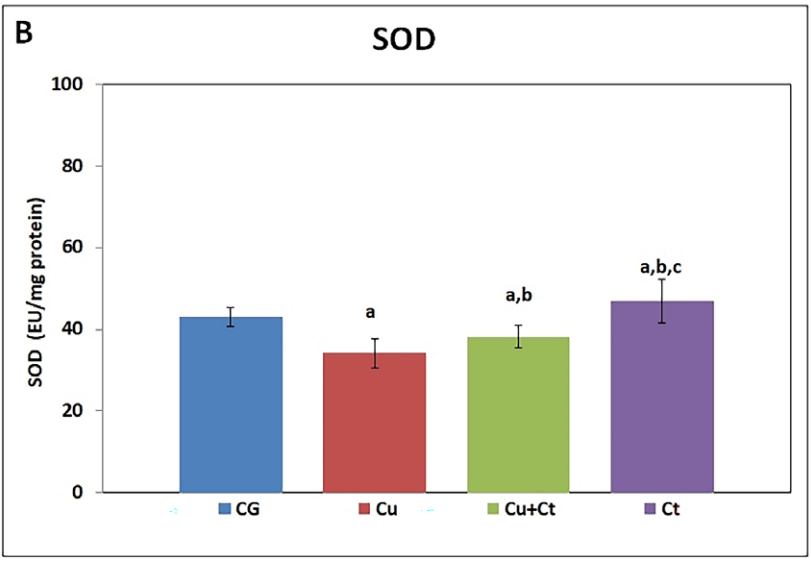
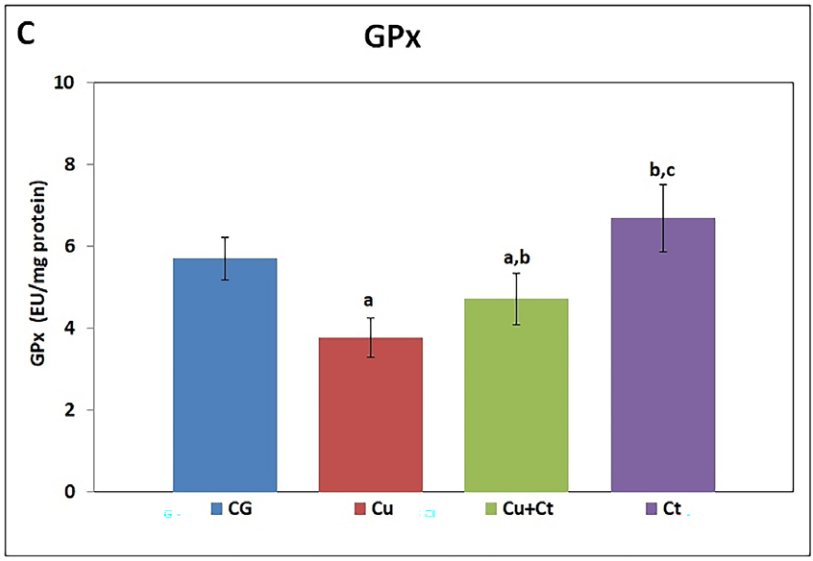


Fig. 2. The MDA content (A), TOS (B), OSI (C) and TAS (D) levels of groups.
MDA: malondialdehyde, TOS: total oxidant status, TAS: total antioxidant status, CG: control group, Cu: copper group, Cu+Ct: copper + *Celtis* group, Ct: *Celtis* group.
[a] - significantly different from the control group ($P < 0$ .05); [b] - significantly different from Cu ($P < 0.05$); [c] - significantly different from the Cu+Ct group ($P < 0.05$).


A
GSH
GSH (nmol/mg tissue)
10
8
6
4
2
0
a
b,c
CG
Cu
Cu+Ct
Ct


B
SOD
SOD (EU/mg protein)
100
80
60
40
20
0
a
a,b
a,b,c
CG
Cu
Cu+Ct
Ct



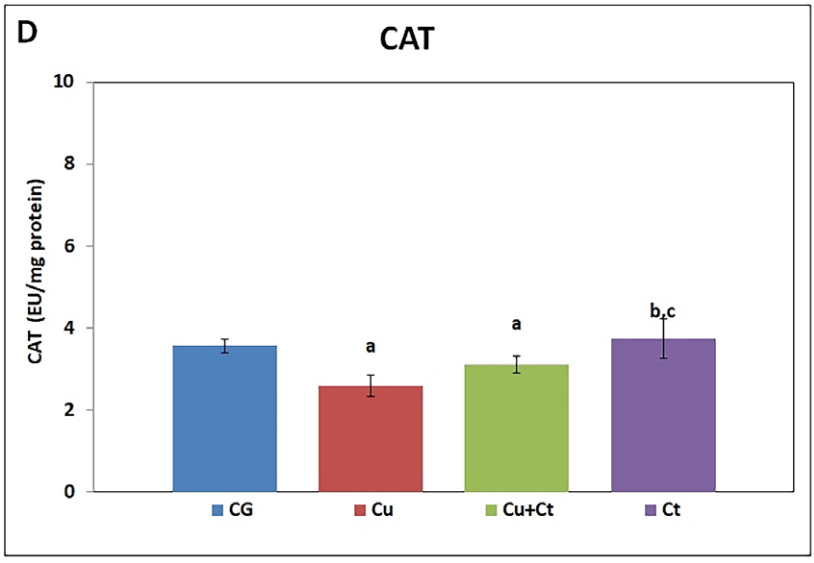


Fig. 3. The GSH content (A) and SOD (B), GPx (C), CAT (D) activities of groups.
GSH: glutathione, SOD: superoxide dismutase, GPx: glutathione peroxidase, CAT: catalase, CG: control group, Cu: copper group, Cu+Ct: copper + *Celtis* group, Ct: *Celtis* group.
[a] - significantly different from the control group ($P < 0.05$); [b] - significantly different from the Cu group ($P < 0.05$); [c] - significantly different from the Cu+Ct group ($P < 0.05$).

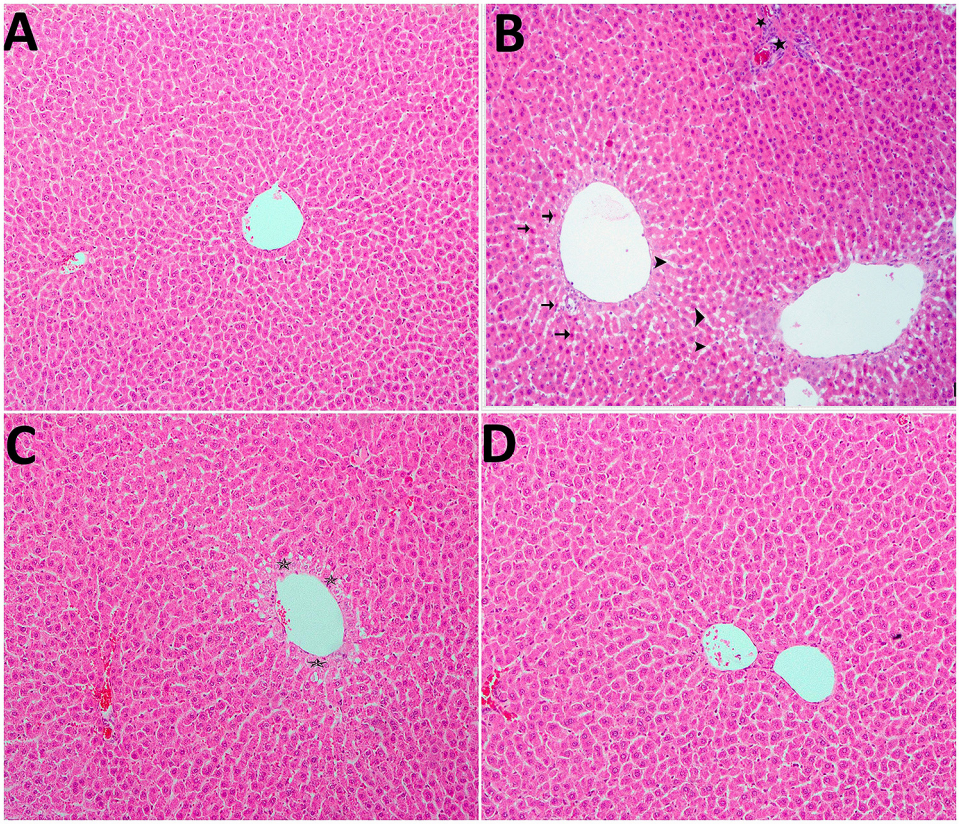


Fig. 4. A: control group, normal histological appearance of the liver; B: Cu group, disseminated centrilobular hepatocellular degeneration and coagulation necrosis (arrows), inflammatory cell infiltration in the portal area (asterisk), dilatation in sinusoid (arrow-heads); C: Cu+Ct group, centrilobular hepatocellular degeneration (asterisk); D: Ct group, normal histological appearance of the liver. H+E × 10. Cu: copper group, Cu+Ct: copper + Celtis group, Ct: Celtis group.