Investigation of The Biochemical, and Histopathological Effects of Alpinia Officinarum Rhizome Extract Against Cisplatin-Induced Hepatotoxicity in Rats

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Abstract

This study aimed to evaluate the effect of *Alpinia officinarum* rhizome extract (AORE) on cisplatin (CP)-induced hepatotoxicity in rats. In this regard, 44 male rats were divided into six groups including the control group, AORE control group, CP control group, and three groups of CP (7 mg/kg dose, on the tenth day) with AORE (at concentrations of 100, 200 and 400 mg/kg, daily for 14 days). After 14 days, the rats' livers were removed and their liver function was assessed using biochemical marker enzymes. Oxidative stress was assessed by evaluating malondialdehyde concentration and hepatic superoxide dismutase activity and a histopathological test was also conducted. According to the results, the group treated with CP had severe liver damage and the group treated with AORE had no harm compared with the control group. Among the groups receiving CP and AORE (at different concentrations), the group with 200 mg/kg AORE showed the lowest liver damage compared to the other two groups.

Introduction

It is known that cancer is one of the most important health issues around the world (Yang 2019 ; Toğaçar 2020 ; Nouri 2020 ; Dizaji 2020). Various methods and drugs have been developed for to confront this issue (Nouri 2020 ; Rouhani 2018 ; Sharifianjazi 2020 ; Asadi 2019; Bazli 2017; Bazli L 2020 ; Jang 2020 ; Radmansouri 2018 ; Abasian 2019). One of the powerful chemotherapeutic agents used for a wide range of malignancies such as ovarian, bladder, neck, cervical, and testicular cancers is cisplatin (CP) (Zhao 2019; Sun 2019).

. Although this substance plays an important role in different cancer treatments, several negative impacts have been reported such as hepatotoxicity, ototoxicity, renal dysfunction, nausea, and vomiting (Poon 2009). It has also significant side effects on the kidney, however, it is metabolized by the liver (Townsend 2009; Yimit 2019 ; Ghosh S 2019). According to histopathological investigations, severe damages can be induced to the liver by CP such as degeneration of hepatocytes and it can moderate the sinusoid dilatation (Lynch 2005; Miyamoto 2007). To alleviate the adverse effects of CP, antioxidant treatment has been used. Nephrotoxicity and hepatotoxicity have been shown to stem from oxidative stress produced by CP(Chirino 2009 ; Sezen 2008 ; Yüce 2007).

The medicinal plant of *Alpinia officinarum* mostly grows in the tropical and subtropical regions of Southeast Asia. This is a kind of Chinese medicinal material, which is consumed as a food additive. *Alpinia officinarum* Hance is obtained from the rhizome of ginger family, Zingibriaceae, and has a pleasant and pungent odor. Due to its high safety, it has been registered by the National Health and Family Planning Commission of the People's Republic of China as an affinal drug and diet (Zhao 2019; Pillai 2018; Zhang 2020). It has been found that *Alpinia officinarum* possesses anti-inflammatory (Rajendiran 2018), antibacterial (Huang 2008; Pillai 2019), anticancer (Ghil S 2013; Awaad 2018) , and antihyperlipidemic properties(Pillai 2018; Lin 2015).Previous studies have identified bioactive compounds of *Alpinia officinarum* rhizome extract (AORE). Higher content of the compounds exists in the rhizomatous extract of *Alpinia officinarum* compared to those found in the aerial parts. These bioactive

compounds include linear, dimeric, and cyclic diarylheptanoids, volatile oils, flavonoids, lignin, phenylpropanoid, and diterpenoids (Zhang 2016). The extract containing the bioactive compounds exhibit antitumor effects in various cancerous cell lines such as breast, lung, neuroblastoma, and liver (SA 2018 ; Hu 2019 ; Zhang 2019) Abbas et al(Abass et all 2018) developed a model based on hepatocellular carcinoma with and without CP to study the sensitizing and prevention efficiency of AORE. It was reported that AORE is able to act as a potential natural chemosensitizing or chemoprevention agent against hepatocarcinogenesis.

Although there are several studies about the effect of AORE on preventing hepatotoxicity of CP, the biochemical mechanism or cellular response by which AORE protects the liver against CP-induced damage has not been investigated. We hypothesized that AORE prevents CP-induced hepatotoxicity due to having inherent antioxidant potential and improves metabolism and antioxidant defense mechanisms of the liver. The aim of this study was to evaluate the biochemical, and histopathological effects of AORE in rats against CP-induced hepatotoxicity.

Materials And Methods

Preparation of AORE

Alpinia officinarum rhizome was procured from the local market in December 2019 (Bushehr, Iran). The plant was washed twice with distilled water to remove any pollution and air-dried at room temperature for 24 h. Then, the freshly dried rhizomes were ground to obtain a fine powder. For the preparation of hydro-alcoholic extraction, dried rhizomes were mixed with ethanol (95% v/v). The mixture was filtered by a Whatman filter paper (No. 4). The hydro-alcoholic extract was concentrated using a rotary evaporator under vacuum at 50 °C for 10 min. AORE was prepared in different concentrations of 100, 200, and 400 mg/kg. The extract was stored in a refrigerator at 4 °C prior to experimental trials (Lee 2009 ;Mazaheri 2014).

Animals

Forty-eight Wistar albino male rats (250-300 g) were procured from the animal house of the School of Medicine. The rats were maintained under a standard laboratory condition with thermally controlled temperature (20-25 °C), automatically illuminated room (12 h light: 12 h darkness) for one week. They were kept under proper diet and good ventilation during the experiment and they had free access to food and water. All experimental protocols were approved by the Institutional Animal Care and Ethics Committee of the Medical science of Jundishapur University (Ahvaz, Iran).

Experimental design

The rats were randomly divided into 6 groups (n=6 for Group I and Group II and n=8 for other groups): Group I (control); rats received normal saline by gavage for 14 days and received a single intraperitoneal injection of normal saline on the 10th day. Group II ($CP^-/AORE^+$); rats received just AORE (400 mg/kg)

daily by oral administration for 14 days without any injection of CP. Group III ($CP^+/AORE^-$); rats received normal saline by gavage for 14 days and received a single intraperitoneal injection of CP (7 mg/kg) on the 10th day. Group IV ($CP^+/AORE^+100$); rats received AORE (100 mg/kg) for 14 days and CP (7 mg/kg) on the 10th day. Group V ($CP^+/AORE^+200$); rats received AORE (200 mg/kg) for 14 days and CP (7 mg/kg) on the 10th day. Group VI ($CP^+/AORE^+400$); rats received AORE (400 mg/kg) for 14 days and CP (7 mg/kg) on the 10th day. In groups that received both CP and AORE, AORE was administered one hour before CP treatment. On the 14^{th} day, biochemical, histopathological, and immunohistochemical assays were carried out. The entry and exit criteria were male rats, without receiving any medication before the study initiation and the use of healthy rats without anatomical abnormality.

Specimen collection

Xylazine (5 mg/kg) and ketamine (50 mg/kg) were utilized to anesthetize the rats after 14 days. Blood samples were taken from the heart of each rat. To evaluate serum liver enzymes, serum was separated from the blood. Thereafter, the rats' livers were removed immediately after sacrificing the rats. A part of the liver was washed using PBS and after weighing was freshly used for biochemical evaluations. The remaining tissue was fixed in 10% buffer formalin for immunohistochemical and histological assays (Amiri 2018).

Evaluation of liver function

The biochemical markers of liver damage, including serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), albumin, total protein, and total bilirubin (T. bilirubin) levels were determined using Pars Azmoon kits (Tehran, Iran) according to the manufacturer's instructions.

Evaluation of oxidative markers

Malondialdehyde (MDA) concentration in liver homogenate was measured based on the reaction of thiobarbituric acid with MDA to form thiobarbituric acid reactive species (TBARs). The measurements were conducted colorimetrically at 534 nm and relative to MDA content similar to previous studies (Puntel 2007; Nelson 1993; Ohkawa 1979). using ZellBio kits (Germany).

Evaluation of antioxidant activity

Hepatic superoxide dismutase (SOD) activity was determined based on the ability of the enzyme to inhibit phenazine methosulfate-mediated reaction of nitroblue tetrazolium. The measurement was carried out colorimetrically at 560 nm according to previous studies (Lee 2009 ;Mazaheri 2014) using ZellBio kits (Germany).

Histopathological analysis of liver tissue

For histopathological assay and to determine the effect of CP and AORE on the liver, samples were fixed in 10% (w/v) buffer formalin for 24 hours. The standard protocol was used to process and embed the tissue in paraffin and prepare sections with a thickness of 5 µm. The sections were then stained with eosin and hematoxylin and evaluated using a light microscope with a 40× magnification by a histologist who was blinded to the groups and the corresponding treatments. Histological photomicrographs converted to quantitative analysis using a scoring system (Amiri 2018(. Based on the extent of cytoplasmic vacuolization, inflammatory cell infiltration, sinusoidal dilatation, degeneration, and congestion, the results scored as 0, 1, 2, and 3 representing normal, mild, moderate, and severe damage, respectively.

Statistical analysis

SPSS 19 (Chicago, USA) was employed for statistical data analysis. The measured data were reported as the mean value ± SD. The comparison among the groups was performed by one-way ANOVA followed by the Post hoc Tukey test and statistical significance was considered to be $P < 0.05$.

Results

Liver biochemical marker enzymes

The levels of the biochemical marker enzymes for liver damage, including AST, ALT, ALP, and LDH for different groups are shown in Fig. 1. According to the results, the $CP^-/AORE^+$ group showed no significant differences with the control group, whereas the level of the markers significantly enhanced ($P<0.001$) in the $CP^+/AORE^-$ group in comparison to the control group. The level of biochemical enzymes in the $CP^+/AORE^+100$ group showed a significant increase compared to the control group ($P<0.001$). The level of AST and ALP enzymes in this group exhibited a significant decrease in comparison with the group treated with $CP^+/AORE^-$ ($P<0.001$ and $P<0.05$, respectively).

Levels of chemical enzymes in the $CP^+/AORE^+200$ group showed a significant reduction compared to the $CP^+/AORE^-$ and $CP^+/AORE^+100$ groups ($P<0.001$). This group showed a significant enhancement in ALT enzyme level compared to the control group ($P<0.001$) but did not differ from the control group in other enzyme levels. The level of the enzymes in the $CP^+/AORE^+400$ group showed a significant decrease in comparison with the $CP^+/AORE^-$ and $CP^+/AORE^+100$ groups ($P<0.001$). In this group, a significant rise in the level of ALT ($P<0.001$), ALP ($P<0.001$), as well as AST ($P<0.05$) was observed compared to the control group. Even the ALT enzyme level was significantly enhanced compared to the $CP^+/AORE^+200$ group ($P<0.001$).

Data represented in Table 1 show the serum total bilirubin, protein, and albumin levels. According to the results, the level of bilirubin in the $CP^+/AORE^-$ group increased compared to the control group, while the level of protein and albumin was reduced significantly compared. There was a statistically significant difference between the results of this group and the control group. The level of these serums in the

$CP^-/AORE^+$ group did not change noticeably compared to the control group and the difference between the results of the two groups was not significant.

Serum bilirubin level in the $CP^+/AORE^+100$ group enhanced significantly compared to the control group and there was a significant difference in the results, but compared to the $CP^+/AORE^-$, $CP^+/AORE^+200$, and $CP^+/AORE^+400$ groups, significant changes were not observed and there was no statistical difference between their results. The serum protein level in the $CP^+/AORE^+100$ group was reduced compared to the control group and there was a statistically significant difference between the results, while compared to the $CP^+/AORE^-$, $CP^+/AORE^+200$, and $CP^+/AORE^+400$ groups, there was no significant difference between the results. The results of serum albumin levels in this group showed similar results to total serum protein, however, the serum albumin level of group $CP^+/AORE^+200$ was higher than that of the $CP^+/AORE^+100$ group and there was a significant difference between their results.

The level of serum bilirubin in the $CP^+/AORE^+200$ group increased significantly compared to the control group and there was a significant difference in the statistical results, but compared to the $CP^+/AORE^-$ group, it decreased with a statistically significant difference. The serum protein level in group $CP^+/AORE^+200$ did not change remarkably relative to the control group and there was no statistically significant difference between the results. The result of the serum albumin level was similar to that of the total serum protein.

The serum bilirubin level in the $CP^+/AORE^+400$ group rised significantly compared to the control group, however, it showed a decrement compared to the $CP^+/AORE^-$ group. The differences were statistically significant. Furthermore, the level of serum protein in the $CP^+/AORE^+400$ group decreased relative to the control group and there was a statistically significant difference between the results, but there was no significant change and no statistical difference between the results of this group and the $CP^+/AORE^-$ group. The results of the serum albumin level for this group were also similar to those of total serum protein.

Oxidative stress

As shown in Table 2, the $CP^+/AORE^-$ group showed a significant increment in the level of MDA. According to the result of the $CP^-/AORE^+$ group, the MDA level was diminished relative to the control group. All groups showed a significant difference with the control group (P <0.05). Moreover, the $CP^+/AORE^+200$ group showed a lower liver MDA level than did $CP^+/AORE^+100$ and $CP^+/AORE^+400$. The difference between the result of $CP^+/AORE^+100$ and $CP^+/AORE^+400$ was not significant.

The SOD activity of the $CP^+/AORE^-$ group was significantly reduced in the liver tissue compared to the control group. On the other hand, an increment in the SOD activity of the $CP^-/AORE^+$ group was not significant in comparison with the control group. The $CP^+/AORE^+400$ group demonstrated a lower SOD

activity level compared to the $CP^+/AORE^+100$ and $CP^+/AORE^+200$ groups in the liver tissue. A significant enhancement of the SOD activity was exhibited in the $CP^+/AORE^+200$ compared to the $CP^+/AORE^-$ group.

Liver histopathological study

The photomicrographs of the liver tissue in different groups are presented in Fig. 2. The control group (Fig. 2a) showed normal histoarchitecture of lobules in the liver (sinusoids, hepatocytes, and Kupffer cells). No change in the liver structure was observed in the rats treated with AORE ($CP^-/AORE^+$) (Fig. 2b). Fig. 2c illustrates a liver tissue from the $CP^+/AORE^-$ group. Administration of CP resulted in disorganization in lobules such as dilation sinusoids, congestion, hemorrhage, periportal leukocyte infiltration, hepatocyte vacuolization, enlarged nuclei, and focal necrosis with pyknotic (Score 3). On the other hand, the groups treated with CP and AORE (CP^+/AO^+100, $CP^+/AORE^+200$, and $CP^+/AORE^+400$) exhibited little hepatic damage (Score 1) in comparison with those treated with CP alone (Fig. 2d-2f). The damage in these groups was determined as slight hydropic degeneration of hepatocytes and congestion of blood sinusoid. However, lower hepatic damage was observed in the $CP^+/AORE^+200$ group relative to the $CP^+/AORE^+100$ and $CP^+/AORE^+400$ groups. The damage severity in all groups have been listed in Table 3 based on the defined scoring.

Discussion

CP-induced hepatotoxicity is commonly characterized by the changes in biochemical, molecular, and histological parameters (Ekinci 2020). Specific liver enzymes can be measured to determine hepatotoxicity. As liver enzyme activities are much higher than those of serum (about 1000 times), the serum enzyme activity will be doubled in case only 1% of hepatocytes necrotize occurs (Mir 2015). In this study, the levels of AST, ALT, LDH, and ALP, as the biochemical marker enzymes of damaged liver tissue, were measured to study the effect of AORE in the prevention of CP-induced hepatotoxicity. An increment in the enzyme levels is the result of losing functional integrity of hepatocyte cell membrane in response to adverse effects of some drugs as well as pathological conditions as cirrhosis leading to the leakage of these enzymes (Kadir 2013). In this study, the levels of the biochemical marker enzymes were the highest in the group that received CP compared to other groups. CP has been found to be accumulated in the liver leading to some toxic activities and increasing the hepatic cell permeability or provoking cellular destruction (Mansouri 2017).

The measurement of other liver parameters exhibited that serum total albumin and protein levels of the CP-treated group decreased relative to the control and $CP^-/AORE^+$ groups, while the total bilirubin level enhanced. The increment in the total bilirubin level has been reported to be due to defense mechanisms against oxidative injury induced by free radicals. These mechanisms involve the reduction of free radicals by increasing the level of electron donors such as bilirubin (Hozayen 2014). Poor health and worse consequences can be inferred from the low levels of total protein (Ibrahim 2008). The alteration of the serum levels of bilirubin, albumin, protein, and the hepatic enzymes indicated impaired liver function in

the $CP^+/AORE^-$ group. Similar results have been reported in previous studies. It has been indicated that the level of ALP, LDH, ALT, AST, and serum total bilirubin increased and the albumin and protein levels decreased in CP-treated groups showing CP-induced hepatotoxicity (İşeri 2007; Al-Malki 2014).

The liver function indices improved in the groups administered by AORE only or combined with CP. Similar results were obtained for the control group and the $CP^-/AORE^+$ group and the best results were observed in the $CP^-/AORE^+200$ compared to other doses of AORE. The hepatoprotective effect of AORE might be due to the presence of antioxidants, bioactive phytochemicals such as flavone and flavonoids (Abbas 2018). Rajesh et al (Rajesh 2013) reported an enhancement in the serum hepatic enzyme levels in ischemic reperfusion rats treated by 200 mg/kg and 400 mg/kg of AORE. In another study by Xia et al. (Xia 2010). the ALT activity was similar to that of the control group in rat models administered by AORE. It was also reported by Abbas et al. (Abbas 2018) that using 400 mg/kg of AORE led to the improvement of the liver function indices.

In this study, liver damage caused by CP was also determined by the increase in MDA besides the reduction of hepatic antioxidants such as SOD activity. Potential oxidative stress induction by CP injection which results in cell death in liver tissue was reported by previous studies (Ekinci 2020;Hassan 2020 ; Choi 2019 ;Soares 2013; Ekinci 2017). Oxidative stress is caused by the overproduction of reactive oxygen species and/or antioxidant mechanism failure (Karale 2017). In normal metabolism, produced reactive oxygen species are removed in the liver through specific reactions in which some enzymes including catalase, SOD, peroxiredoxin, and glutathione peroxidase (GSH Pxs) are involved. In the case of oxidative stress exposure like CP-induced hepatotoxicity, a large volume of reactive oxygen species beyond the detoxification capacity is produced in liver cells. Therefore, hepatocyte injury is caused by DNA damage (reduced proliferation and promoted cell death), lipid peroxidation (radical formation and inflammation), protein oxidation (reduced enzymatic functions and growth factor inhibition), as well as a direct effect on signal pathways (reduced NO level, reduced angiogenesis, and transcription factor activation) (Bilgic 2018).

The treatment of the groups with AORE led to a noticeable decrement in the hepatic MDA content together with an increment in hepatic SOD activities. Similar performance was observed in the control and $CP^-/AORE^+$ groups and the $CP^+/AORE^+200$ exhibited the best results in liver function indices. The antioxidant effect of AORE is stemmed from flavonoid antioxidant compounds (Sani 2019; Tungmunnithum 2020). The flavonoid (galangin) has free-radical scavenging activities resulting in suppression of the genotoxicity of chemicals and modulation of enzyme activities (Swain 2020).

According to the histopathological studies, the liver structure of the $CP^-/AORE^+$ group was similar to that of the control group, while hepatic damage was observed in the $CP^+/AORE^-$ group. Treatment with AORE led to the alleviation of liver injury in rats. The results are in good agreement with other histopathological investigations revealing the incidence of less damage to the liver tissue in AORE-treated rats in comparison with reperfusion/hepatic ischemia-induced damage in rats (Rajesh 2013)

Conclusions

To summarize, the positive impact of the AORE administration on the improvement of hepatotoxicity in rats induced by CP was demonstrated. This is conducted through a number of intracellular pathways such as improving liver function parameters, and suppressing oxidative stress, as well as modifying antioxidant defense mechanisms. The rise in the levels of ALT, AST, LDH, ALP, and serum total bilirubin as well as the reduction of serum total protein and albumin were the determining factors for hepatic injury, which was remarkably improved in AORE-treated groups. Moreover, AORE treatment resulted in a reduction in SOD and an increase in MDA. In the histopathological examination of AORE-treated groups, significantly fewer hepatocytes damage was observed. Overall, the strongest antioxidant properties were shown in the CP^{+}/$AORE^{+}$200 group. The results may modify cancer treatment protocols to include phytoceuticals to render safer cancer treatments.

Declarations

Ethics approval and consent to participate

All institutional and national guidelines for the care and use of laboratory animals were followed.

Consent for publication

Not applicable' for that specific section.

Availability of data and materials

The data that support the findings of this study are available on request from the first author or corresponding author upon request.

Competing interests

The authors declare that they have no conflict of interest.

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Authors' Contributions

FN performed the histological examination of the Liver, and was a major contributor in writing the manuscript. AA participated in animal interventions and drug therapy phase and was a major contributor in writing the manuscript. MA participated in conducting experiments and writing the article. NC analyzed

and interpreted the data regarding the biochemical assay. All authors read and approved the final manuscript.

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Tables

Table 1 The levels of serum total bilirubin, serum total protein, and serum albumin in different studied groups

Groups	Bilirubin (g/dl)	Total Pr (g/dl)	Serum Alb (g/dl)
Control	.24±0.03^{c}	6.70±0.56^{a}	3.24±0.08^{a}
$CP^{-}/AORE^{+}$	0.15±0.05^{c}	6.91±0.21^{a}	3.34±0.12^{a}
$CP^{+}/AORE^{-}$	1.875±0.17^{a}	5.72±0.09^{b}	2.99±0.09^{bc}
$CP^{+}/AORE^{+}100$	1.85±0.12^{ab}	5.53±0.35^{b}	2.82±0.11^{c}
$CP^{+}/AORE^{+}200$	1.35±0.23^{b}	6.2825±0.36^{ab}	3.23±0.05^{ab}
$CP^{+}/AORE^{+}400$	1.55±0.12^{b}	5.54±0.39^{b}	2.93±0.16^{c}

At each column, disparate lowercase letters show a significant difference (p <0.05) between groups.

Table 2 Liver homogenate MDA contents and SOD activities among different studied groups

Groups	MDA (nmol/g.tissue)	SOD (U/g.tissue)
Control	62.17±3.44^{a}	85.93±3.25^{a}
$CP^{-}/AORE^{+}$	58.40±1.87^{b}	86.64±2.97^{a}
$CP^{+}/AORE^{-}$	119.12±3.06^{c}	47.87±1.63^{b}
$CP^{+}/AORE^{+}100$	91.12±1.45^{d}	55.63±2.04^{c}
$CP^{+}/AORE^{+}200$	80.60±1.45^{e}	62.55±1.75^{d}
$CP^{+}/AORE^{+}400$	91.71±0.94^{d}	53.42±1.09^{c}

At each column, disparate lowercase letters show a significant difference (p <0.05) between groups.

Table 3 Severity of histopathological damage in the rats' liver

Groups	Number of rats	Score			
		0	1	2	3
Control	6	6	0	0	0
$CP^-/AORE^+$	6	6	0	0	0
$CP^+/AORE^-$	8	0	1	2	5
$CP^+/AORE^+100$	8	0	5	3	0
$CP^+/AORE^+200$	8	0	7	1	0
$CP^+/AORE^+400$	8	0	6	2	0

Figures

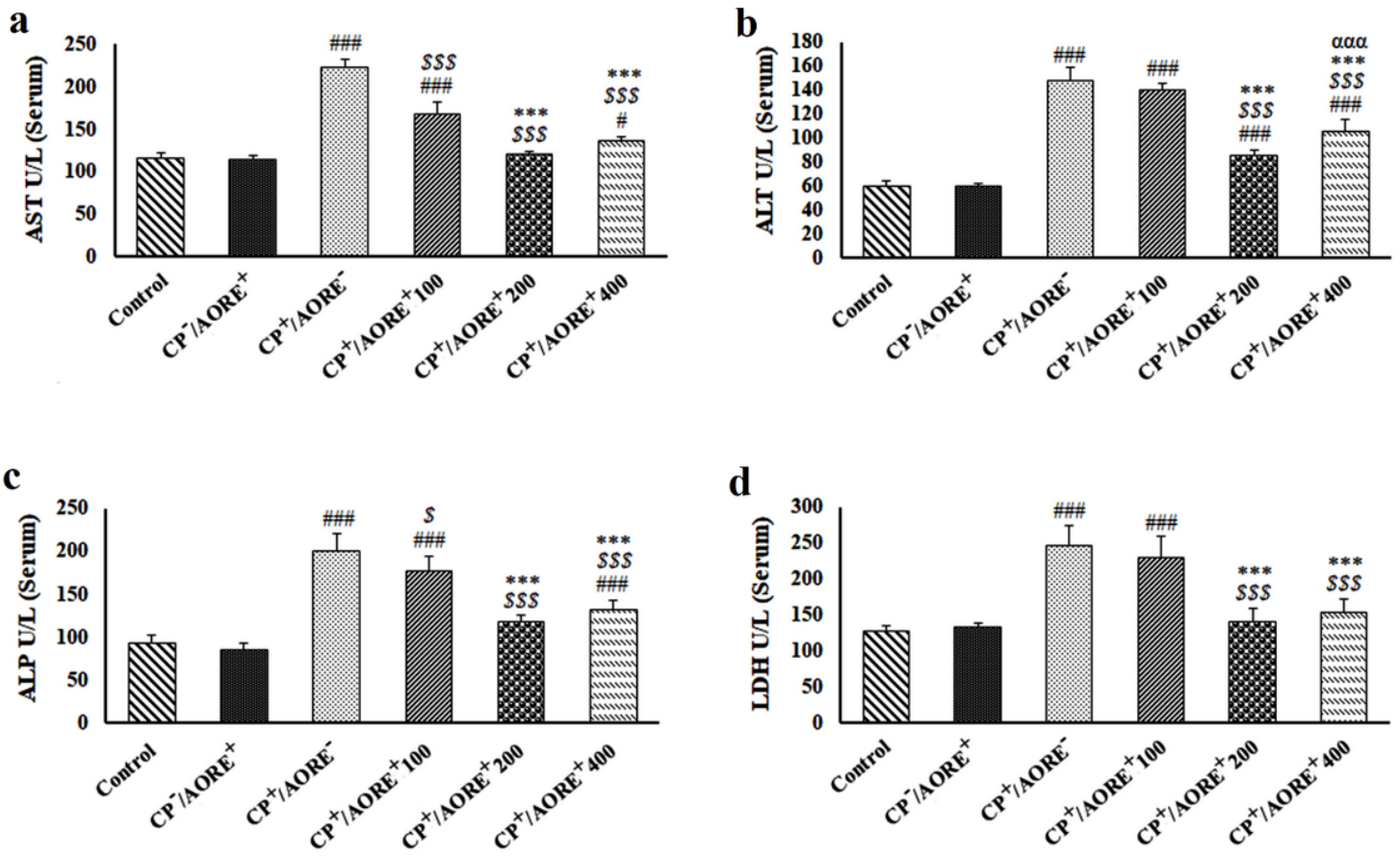

Figure 1

The levels of the biochemical marker enzymes for liver damage in different studied groups. a: AST, b: ALT, c: ALP, and d: LDH. Data are means ± SD. ###p < 0.001 vs. control group; $, p < 0.05; $$$, p < 0.001 vs.

the CP+/AORE- group; ***, p < 0.001 vs. the CP+/AORE+100 group; ααα, p < 0.001 vs. the CP+/AORE+ 200 group

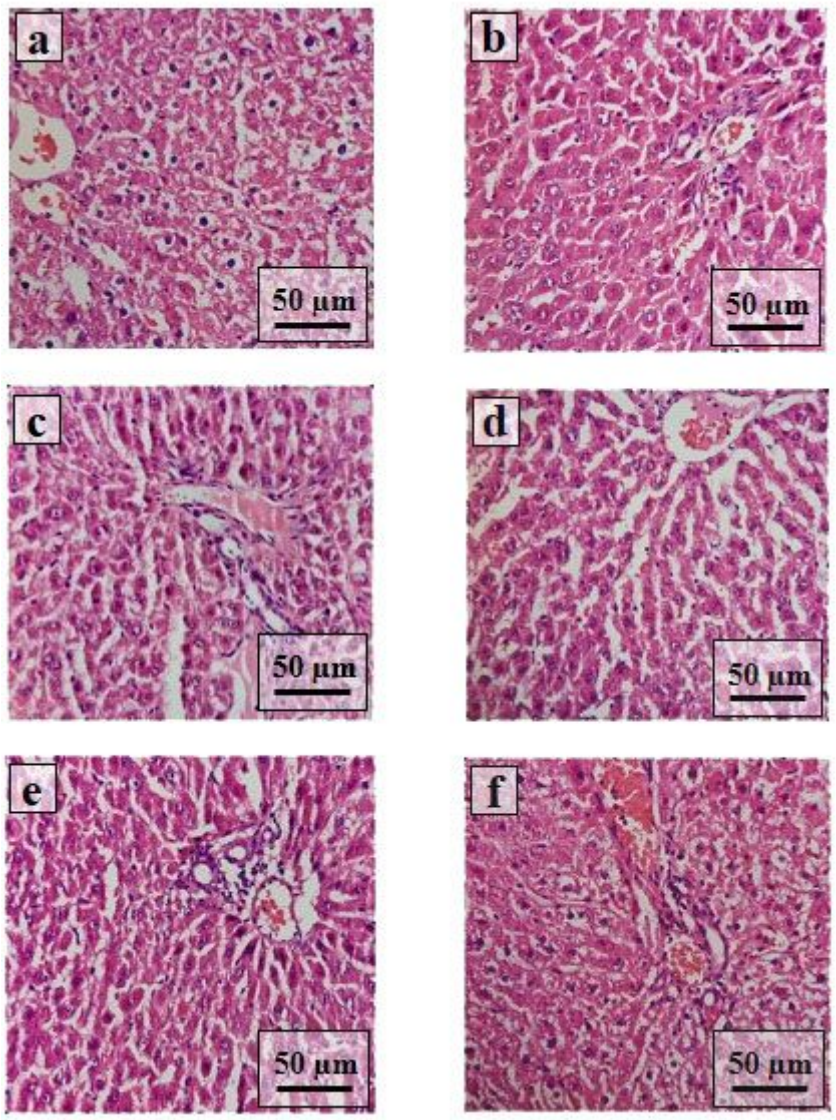

Figure 2

Photomicrographs of the liver tissue in rats. a: Control, b: CP-/AORE+, c: CP+/AORE-, d: CP+/AORE+100, e: CP+/AORE+200, f: CP+/AORE+400 (H & E Staining)