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Protective efficacy of Allium jesdianum on liver parameters, inflammatory cytokines, oxidative injury and apoptotic changes in mercuric chloride-induced hepatotoxicity in rats

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SUMMARY

Mercuric chloride (MC) is a chemical compound made from a combination of mercury and chlorine causing intracellular oxidative stress generation. Allium jesdianum (AJ), as a member of the Liliaceae family, has various pharmacological and strong antioxidant properties. This study aimed to evaluate the probable therapeutic effects of AJ against hepatocytes degeneration, inflammation, apoptotic changes and oxidative injuries induced by MC administration. Sixty-four rats were randomly divided in eight groups (n = 8) including groups of control, MC (50 mg/kg), AJ (500, 1000, 2000 µg/ml), and MC+AJ. They were intraperitoneally and orally administrated for one week. Nitrite oxide, lipid peroxidation (LP) levels, and Ferric Reducing Ability of Plasma (FRAP) assays were conducted to evaluate the intracellular antioxidant index.

The level of apoptotic genes expression (p53, Bcl2 and Bax) were assessed by real-time PCR. Cytokines involved in systemic inflammation were evaluated by ELISA. Hepatic enzymes activity was also measured. Microscopic histopathological assessments (hepatocytes dimeter (HD) and central hepatic vein (CHV)), and apoptosis cell index were also analyzed by light and fluorescent microscopy methods. MC treatments were significantly increased, all parameters described in methods section (except FRAP level and Bcl2, which was decreased) in MC group, compared to the control group ($P < 0.05$). Furthermore, all indices parameters were significantly reduced in AJ and AJ + MC groups (except FRAP level and Bcl2, which was increased) as compared to the MC group ($P < 0.05$). Our findings revealed that AJ effectively reduce hepatotoxicity induced by MC administration through the activation of antioxidant pathways and

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regeneration of histopathological alterations.

Key words: Allium Jesdianum – Mercuric chloride – Oxidative – Apoptosis – Hepatotoxicity – Inflammation

INTRODUCTION

The application of secondary defense systems like antioxidant agents can eliminate inflicted cellular injuries. Plant-based antioxidant elements, in addition to excellent therapeutic features (Salahshoor et al., 2020), also have minus harmful side-effects on cells. The AJ plant, with accepted pharmacological properties, is grown in the Middle East regions (Kalantari et al., 2018a). AJ is a prolific source of natural secondary metabolites and antioxidant agents such as flavonoids and phenolic compounds. These chemical agents exert antioxidant properties and activate white blood cells in response to superoxide produced via NADPH oxidase (Naeini et al., 2020). Besides, this plant possesses a wide array of therapeutic potentials and uses such as a traditional medicine for acute gastrointestinal and rheumatic pains (Sohrabinezhad et al., 2019).

Enhanced Reactive Oxygen Species (ROS) accumulation in the body induces lipid peroxidation (LP) occurrence, secretion of inflammatory cytokines, and increases nitrite oxide production in various tissues (Salahshoor et al., 2019). Lipid peroxidation is the main element involved in oxidative injury of lipid structures, lipoproteins, and cell membranes (Zhu et al., 2019). Cellular LP process causes generation and accumulation of hydroxides and peroxides, which are the bases of cell toxicity and reduced function of antioxidant enzymes in the body (Salahshoor et al., 2018).

MC ($HgCl_2$) is composed of chlorine and mercury (Ansar and Iqbal, 2016). MC has a yellowish or bright white color with extensive application in chemistry laboratories. It was also used for syphilis treatment. However, today it is not consumed anymore due to mercury poisoning (Koli et al., 2019). MC damages the cells, DNA, and cell membrane leading to cell death (Betti and Pool, 1993). Some elements, such as increased levels of inflammatory cytokines, ROS, and nitrite oxide are thought to complicate MC harmful effects (Ahmad and Mahmood, 2019). The exposure to MC causes oxidative stress production, such as H_2O_2 and OH, stimulation of cell destruction through interference with cellular metabolism and membrane lipids damage (Liu et al., 2018). The oxidative stress caused by LP leads to cell membrane damage of hepatocytes and activation of liver enzymes (Jalili et al., 2019a).

The main aim of the present study is to evaluate the probable antioxidant effects of hydro-alcoholic extract of AJ in hepatotoxicity caused by MC administration; the present study was conducted based on histological and biochemical analyses.

MATERIALS AND METHODS

Animals

In this in vivo experimental study, sixty-four male rats (Wistar 20-250 g, 8 weeks) were purchased from the specialized and experimental research center of laboratory animals (Pasteur, Tehran, Iran). The animals were kept in cages under standard animal conditions, including temperature (22 ± 2°C), relative humidity (25-35%), and 12/12 h light/dark cycle, with free access to standard pellet and water. The animals were treated according to ethical standards, and the University Ethics Committee's approved experimental procedure for Care and Use of Animals (IR.KUMS.REC. 1398.751).

Preparation of AJ extract

The AJ plant was purchased from reputable herbal medicine centers and followed by a botanist assessment for verification. After removing impurities and excess components, the aerial part of the plant (stems and leaves) was separated, rinsed with water and dried in shadow (during a five-day period), and coarsely pulverized before extraction. Fifty gr of the ground powder were dissolved in 70% ethanol and kept in a 36℃-water bath for 24 hours. The solution was filtered through Whatman filter paper. The resultant was concentrated under reduced pressure using a rotary evaporator. This extract was kept at -20℃ until use (Salahshoor et al., 2019).

Experimental protocols

The 64 rats were randomly divided into eight groups (n=8) as follows: the control group received intraperitoneal injection of normal saline (the same as treatment groups in volume). The second group (MC group) received a single dose of MC (50 mg/kg) intraperitoneally. The third to fifth groups (AJ administration) received AJ extract with the doses of 500, 1000, 2000 µg/ml orally, respectively (for all 7 days). The sixth to eight groups (MC + AJ) received a single dose of MC (50 mg/kg) in order to induce liver damage followed by administration of AJ 500, 1000, 2000 µg/ml intraperitoneally for 7 days (Kalantari et al., 2018b, c; Ansar et al., 2016).

Dissection of tissue samples

The animals were anesthetized and killed with ether inhalation, 24 h after the last treatment. Blood samples of all groups were collected. Five ml of the blood sample was aspirated from the right ventricle and allowed to clot for 20 min at 37° C. The serum separation was then conducted by centrifugation at 4000 rpm for 10 min. The sera were stored at -70°C until analysis. The liver tissues were dissected, and a fragment of the liver (1×13) was fixed in 10% formalin solution for morphometric and histological examinations

(Salahshoor et al., 2019).

Apoptosis assay

The TUNEL test was performed according to the manufacturer's procedure (Roche, Germany). Paraffin-embedded blocks were prepared using an automatic tissue processor. 5 µm histological slices were cut through a microtome (Leica, Germany), and five slices per rat were selected. The tissues were subjected to deparaffinization. After routine deparaffinization and blocking, the slices were exposed to TdT with DIG. DIG labeling and counterstaining were then carried out. To quantitate the rate of apoptosis, the number of positive cells within the liver was calculated (Salahshoor et al., 2020).

Histopathological and morphometric variations

To assess histological alterations, a section of the right lobe of the liver was fixed in 10% formalin. The tissues were then fixed in paraffin, and thin sections (4 µm) were prepared using a microtome (Leica RM 2125, Germany). The slices were then stained with hematoxylin and eosin. Thereafter, some morphological assessments including full cellular area, hepatocyte outline, maximum and minimum axis, mean axis, and CVH were determined by a light microscope at 40× magnifications. At least 50 cells from the separate region were measured to eliminate the probable measurement bias. All procedures were applied with an investigation microscope coupled with a DP12 Camera (3.34-million pixel resolution) and using the Olysia Bio-software (Olympus Optical Co. LTD, Tokyo, Japan) (Salahshoor et al., 2020).

RNA isolation and real-time polymerase chain reaction

The expression level of apoptotic genes p53 (F: 50-AAGCTCATTTCCTGGTATG-30/R:50-CTGCCACAAGAACTAGAGA-30), Bcl2 (F: 50-TGG GATGCCTTTGTGGAACT-30/R: 50-GAGAC AGCCAGGAGAAATCA-30) and Bax (F: 50-ATGGCGAAATGGAGATGAATA-30/R:50-GCATGGGCATCCTTTAACTC-30) were examined using real-time PCR. All liver tissues were stored in the freezer (-80oC) to prevent tissue degeneration. Total RNA content of liver tissue was extracted using the RNeasy mini kit (Qiagen Co.). cDNA was synthesized from the total RNA extraction by RevertAid™ First Strand cDNA Synthesis Kit according to manufacturer's recommendations (Fermentas). In addition, the DNA samples were treated using DNase set kit (Qiagen). The expression level of apoptotic genes was measured through glyceraldehyde-3-phosphate dehydrogenase primer (F: 50- AAGCTCATTTCCTGGTATG-30/ R: 50- CTGCCACAAGAACTAGAGA-30) as endogenous control by SYBR Green through a comparative method (Nikbakht et al., 2013).

Determination of NO level

The Griess method based on colorimetry approaches was employed to measure the NO levels. Five-hundred µL of serum was deproteinized with zinc sulfate (10 mg). After centrifugation (3000 rpm, 10 min), the equal amounts of Griess reagent (1% sulfanilamide, 0.1% Naphtylethylenediamide in 2.5% phosphoric acid) were also added to the supernatant in 96-well ELISA plates and incubated for 10 min at 37°C. The absorbance was stately at 450 nm with the use of a microplate reader. Nitrite concentrations were considered by a sodium nitrite standard curve (Salahshoor et al., 2018).

LP levels

The level of molecular reaction between thiobarbituric acid and malondialdehyde (MDA) following colorimetry process was considered as the measurement of LP levels. The thiobarbituric acid (TBA) test determines malonaldehyde (MDA) generated due to the oxidation of fatty acids. The frozen sample of the liver tissue was used. First, the tissue was washed with the phosphate-buffered saline (pH 7). Then the tissue was homogenized by an ultrasonic homogenizer in a cold phosphate buffer containing ethylene diamine tetra-acetic acid (EDTA).

Twenty µl of supernatant were mixed within the test tubes. The test tube contained 4 µl of butylated hydroxytoluene, 20 µl of phosphoric acid (1M), and 20 µl of TBA solution. The test tube was incubated for 60 min at 70°C and followed by centrifugation (10,000 rpm, 4 min). 80 µl of the supernatant were poured into spectrophotometer tubes. The produced dye in the commercial kit was read at 532 nm, and the MDA level was stated in nmol/mg protein (Salahshoor et al., 2020).

FRAP technique

Total antioxidant capacity (TAC) of the serum was analyzed by the FRAP technique based on the ability of the plasma to reinstate the ferric. A blue color was made when the complex of FeIII-TPTZ in acidic pH payback to FeII and the absorption at the extreme wavelength of 500 nm were read. The element defining the speed of the FeII-TPTZ and blue stain was merely determined the vitalizing power of the sample. TAC values are strategized via the standard curve (Jalili et al., 2019b).

Hepatic enzymes measurement

The liver homogenate was centrifuged twice (12,000 rpm, 10 min). Then, the supernatant was used for the hepatic enzymes measurement, including Aminotransferase (AST), Alanine Aminotransferase (ALT), and Alkaline Phosphatase (ALP). The ALT and AST were tested based on Reitman and Frankel biochemical methods. The ALP protocol was also determined according to the technique which was set out in the practical research

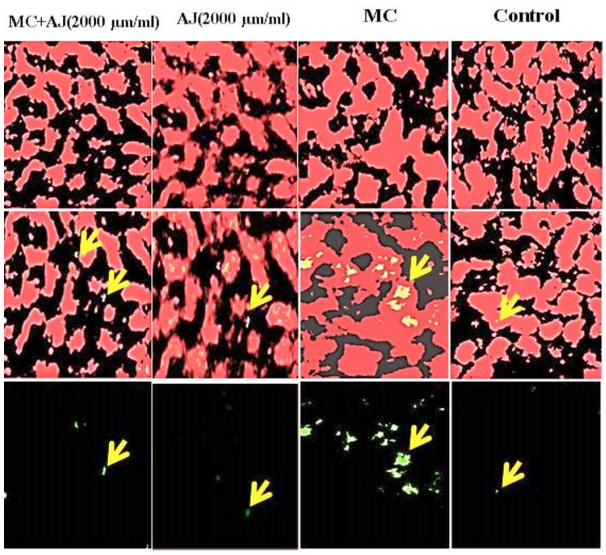

Fig 1. Apoptosis induction in the rat liver following MC and AJ application (200× magnifications, TUNEL staining). Left: Nuclei staining, Middle: cytoplasm staining, Right: Merge. The yellow arrows referred to the shiny green nuclei of apoptotic cells. MC: Mercuric chloride, AJ: Allium Jesdianum.

laboratory (Salahshoor et al., 2019).

Evaluation of inflammation cytokines

The ELISA method was used to assess the Toll-like receptor 4 (TLR4) (My BioSource, California, USA), Interleukin 1 beta (IL-1β) (Abcam Cambridge, UK) and tumor necrosis factor-alpha (TNFα) (Abcam, Cambridge, UK) of the liver. The total proteins of livers extracted were lysed by RIPA (Abcam, Cambridge, UK) and centrifuged at 15,000 g for 30 min. The ratio of 1:20 supernatants/dilutions were seeded into coated microplates with antibodies to induce enzyme- substrate reaction. Standard solutions were used for drawing standard curves. The amounts of proteins were examined in supernatant fractions by ELISA kits. The rate of absorbance was measured in a triplicate manner at 450 nm (Jalili et al., 2019b).

Statistical analysis

The Kolmogorov–Smirnov examination was conducted to confirm the normal distribution of data. One-way analysis of variance (one-way ANOVA) and Tukey post hoc test were applied to statistical analysis and differences among the groups, respectively. The statistical software package of SPSS (Chicago, IL) was used for data analysis. The final results were expressed as mean ± standard error, and $p < 0.05$ was considered as significant.

RESULTS

Apoptotic index

The apoptotic index (AI) was significantly higher in MC group compared to the control group ($P < 0.05$). No significant differences were similarly found in the AI in all AJ groups as compared to the control group ($P > 0.05$). Furthermore, the whole several doses of AJ in AJ and MC+AJ groups represented a significant decline in the AI as compared to the MC group ($P < 0.05$) (Fig. 1 and Table 1).

Morphometric examination

In the experimental groups, a significant incremental effect was detected in the mean diameter of hepatocytes and the CHV among the control and MC groups ($P < 0.05$). Also, in HD and CHV, no significant variations were observed histologically in all AJ groups as compared to the control group ($P > 0.05$). Additionally, the AJ significantly condensed the HD and CHV in all treated members of AJ and MC+AJ groups as compared to the MC group ($P < 0.05$) (Fig. 2).

Histopathological modifications

According to the findings based on histological assessments, the normal liver structure changed in MC control and AJ treatment groups. Following MC administration in the MC group, the liver showed significant histological alterations and hepatic damages, including an increase in irregularities rate, hepatocyte destruction, dilatation of he-

Table 1. Effect of MC and AJ on antioxidant parameters and AI in male rats (n=8 for each group)

Groups	NO (mmol/ml)	FRAP	(mmol/ml)	LP (nmol/mg)	AI (%)
Control	51.75±2.6	134.63±6.4		1.47±00.4	2.1±0.1
MC (50 mg/ kg)	184.45±5.4*	48.72±3.6*		3.82±0.05*	14.3±1.9*
AJ 500 µg/ml	50.39±3.3†	129.14±5.1†		1.27±0.02†	2.4±0.6†
AJ 1000 µg/ml	49.82±2.4†	131.64±6.4†		1.22±0.06†	1.9±0.5†
AJ 2000 µg/ml	50.64±3.8†	128.82±4.7†		1.14±0.04†	2.2±0.9†
AJ+AM 500 µg/ml	112.9+11±7.2¶	75.12±4.6¶		2.13±0.01¶	7.3±0.7¶
AJ+AM 1000 µg/ml	109.18±3.7¶	81.06±4.3		2.06±0.01¶	5.9±0.9¶
AJ+AM 2000 µg/ml	105.26±5.6¶	83.85±6.8¶		1.93±0.02¶	5.8±0.7¶

Data are presented as mean ± SEM. * $P < 0.05$ compared to the control group. † $P < 0.05$ compared to MC group. ¶ $P < 0.01$ compared to the MC group. AI: Apoptotic index, MC: Mercuric chloride, AJ: Allium Jesdianum, NO: Nitrite oxide, FRAP: Ferric reducing the ability of plasma, LP: Lipid peroxidation.

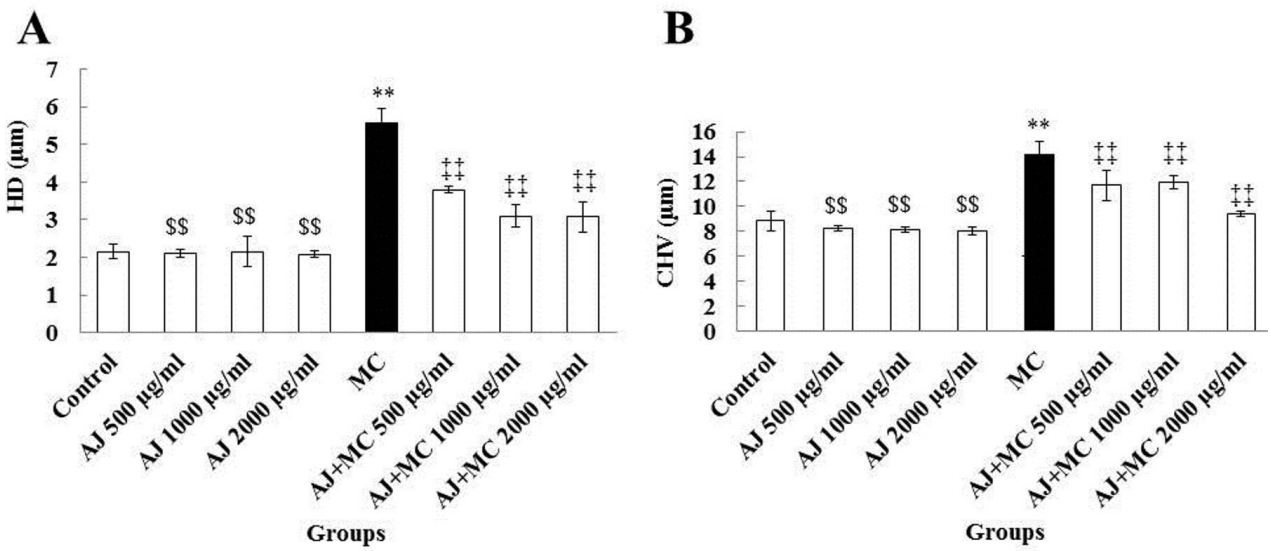

Fig 2. Morphometric alterations of MC and AJ on CHV (**A**) and HD (**B**). **Significant difference as compared to the control group ($P < 0.05$). $$ Significant difference as compared to the MC group ($P < 0.05$). ‡‡Significant difference as compared to the MC group ($P < 0.05$). CHV: Central hepatic vein, HD: Hepatocyte diameter, MC: Mercuric chloride, AJ: Allium Jesdianum.

patic sinusoidal space, hyperemia, and vacuolization of hepatocytes. Treatment with MC + AJ in all doses condensed the hepatic injury caused by MC administration (Fig. 3).

Gene expression levels

Up-regulated changes of apoptotic p53 and Bax genes and down-regulated changes of Bcl2 gene in the group treated with MC compared to the control group were detected as significant (P < 0.05). Also, a significant down-regulation of p53 and Bax genes and up-regulated of Bcl2 apoptotic gene similarly was distinguished in all doses of AJ and AJ+MC groups as compared to the MC group (Fig. 4).

Hepatic levels of FRAP, LP and serum level of nitrite oxide

In the MC group, the FRAP levels were lower than the control group significantly (P < 0.05). Also, in whole groups of AJ and MC + AJ, the FRAP levels were considerably elevated as compared to the MC group (P < 0.05) due to the AJ administration. MC, due to its detrimental effects, significantly increased the nitrite oxide and LP levels in MC group in comparison with the control group (P <

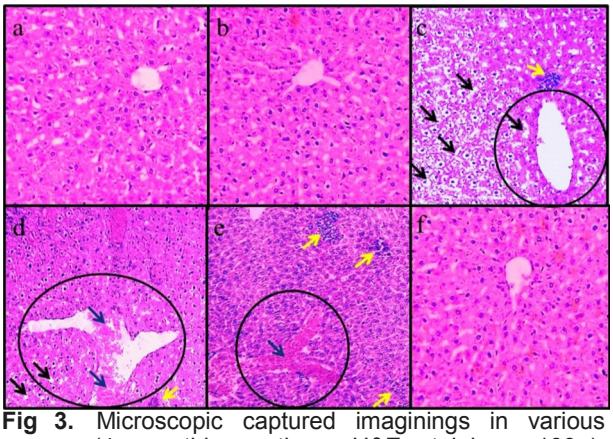

Fig 3. Microscopic captured imaginings in various groups (4 µm thin sections, H&E staining, 100×). Normal liver structure: in control group (a), AJ (2000 µg/ml) group (b), AJ (2000 µg/ml) + MC (50 mg/kg) group (f). Hepatocyte destruction and absence of nuclei in hepatocytes (black arrows), inflammation (yellow arrows), CVH dilatation (circle), and hyperemia (blue arrow), due to the oxidative were recognized in MC group (c, d, e). MC: Mercuric chloride, AJ: Allium Jesdianum.

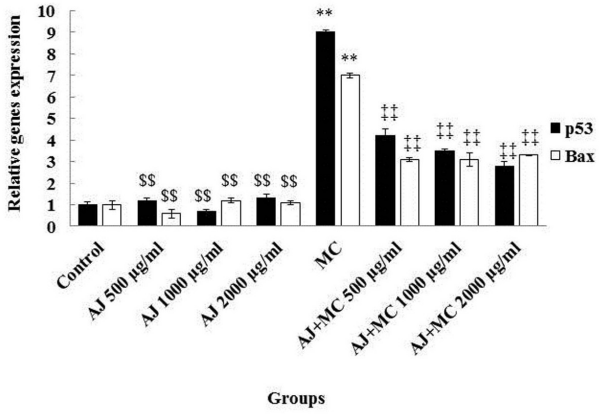

Fig 4. Therapeutics properties of MC and AJ on *p53, Bcl2* and *Bax* genes expression of liver. **Statistically significant ($P < 0.05$) between MC and control group, $$ statistically significant ($P < 0.05$) in MC and MC + AJ groups. ‡‡ Significant modifications in AJ groups as compared to the MC group ($P < 0.05$). MC: Mercuric chloride, AJ: Allium Jesdianum.

Table 2. Effect of MC and AJ on the liver enzymes in male rats (n=8 for each group)

Groups	AST(ng/ml)	ALT(ng/ml)	ALP(ng/ml)
Control	198.36±5.6	64.68±3.6	298.36±7.6
MC (50 mg/ kg)	352.05±7.4*	131.84±5.7*	581.43±8.2*
AJ 500 µg/ml	193.84±4.2†	59.47±4.3†	290.97±5.4†
AJ 1000 µg/ml	190.64±4.4†	59.12±3.7†	295.73±4.8†
AJ 2000 µg/ml	190.15±5.6†	57.34±2.1†	293.14±3.9†
AJ+AM 500 µg/ml	264.71±6.2¶	99.64±4.5¶	381.53±5.4¶
AJ+AM 1000 µg/ml	261.35±4.7¶	95.98±3.2¶	373.77±5.6¶
AJ+AM 2000 µg/ml	158.74±3.1¶	95.17±4.1¶	369.63±6.6¶

Data are presented as mean ± SEM. * P < 0.01 compared to the control group. † P < 0.01 compared to MC group. ¶ P < 0.01 compared to the MC group. MC: Mercuric chloride, AJ: Allium Jesdianum, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase, ALP: Alkaline Phosphatase.

0.05). It is also found that all doses of AJ extract can significantly reduce the mean levels of serum nitrite oxide and LP in the AJ and MC + AJ groups as compared to the MC group (P < 0.05) (Table 1).

Levels of liver enzymes activity

MC administration can lead to a significant increase in liver enzymes levels (ALT, AST, and ALP) in comparison with the control group (P < 0.05). No significant differences were also found in the mean concentration of ALT, AST, and ALP enzymes in all AJ groups compared to the control group (P > 0.05). Besides, whole various doses of AJ in AJ and MC + AJ groups expressed a significant decline in the mean concentration of hepatic enzymes as compared to the MC group (P < 0.05) (Table 2).

Inflammation cytokines

The inflammation cytokines were significantly increased in MC group compared to the control group (P < 0.05). No significant differences were similarly found in the inflammation cytokines in all AJ groups as compared to the control group (P > 0.05). Additionally, whole several doses of AJ in AJ and MC+AJ groups represented a significant decline in the inflammation cytokines as compared to the MC group (P < 0.05) (Table 3).

DISCUSSION

The most vital tissue complicated in the organization of whole-body metabolism is identified as the liver (Salahshoor et al., 2019). Thus, the toxic effects of MC on hepatocytes, oxidative injury, and apoptotic changes were assessed in this study. Besides, the therapeutic antioxidant features of AJ on hepatic injuries were investigated.

In the present study, the AJ showed decreased levels of apoptotic cells and gene expression, including Bax and p53, and also an increased level of Bcl2 gene expression was found. P53 makes the mitochondrial membrane permeable to the influx of cytochrome-c into the intracellular matrix. Accordingly, p53 adjusts the function of apoptotic elements such as Caspase and Bax (Wang et al., 2020). As cell death observed in hepatocytes, it is stated that the MC has up-regulation effects on apoptotic factors (Sugiura et al., 2020). Further, AJ extracts directly translocate within the intra-nuclear space to induce down-regulation of related genes (Zhang et al., 2017). Caglayan et al. (2019) also found that the apoptotic genes are expressed significantly in the kidney after MC administration.

In the current study, the AJ reduced Bax and p53 and improved Bcl2 genes expression in order to prevent cell death. According to the obtained results, it can be indicated that the AJ based on posi-

Table 3. Effect of MC and AJ treatments on the liver levels of TNFα, IL-1β, and TLR4 (n=8 for each group)

Groups	TNFα (pg/ml)	IL-1β (pg/ml)	TLR4(pg/ml)
Control	97.52±2.2	129.55±6.8	48.96±4.8
MC (50 mg/ kg)	172.34±5.1*	284.35±8.3*	135.74±5.3*
AJ 500 µg/ml	93.22±5.3†	131.02±6.9†	49.36±2.7†
AJ 1000 µg/ml	97.04±3.7†	126.85±7.5†	45.08±2.2†
AJ 2000 µg/ml	97.151±2.5†	127.97±3.3†	47.88±4.4†
AJ+AM 500 µg/ml	134.72±4.7¶	144.74±5.5¶	88.44±3.1¶
AJ+AM 1000 µg/ml	131.89±6.2¶	142.66±7.1¶	73.91±2.3¶
AJ+AM 2000 µg/ml	118.04±6.9¶	138.71±6.7¶	75.42±3.8¶

Data are presented as mean ± SEM. * *P* < 0.01 compared to the control group. † *P* < 0.01 compared to MC group. ¶ *P* < 0.01 compared to the MC group. MC: Mercuric chloride, AJ: Allium Jesdianum.

tive antioxidant features affects molecular function and histological construction of hepatocytes. This property causes a diminished level of hepatic enzymes, boosted antioxidant capacity, and reduced NO levels. MC with great mercury content can prompt a varied range of injuries such as liver and heart diseases, allergy, and various side effects on hepatocyte (due to involvement of liver enzymes) (Houston, 2007).

This study confirmed the morphological changes following MC administration including a significant increase in hepatocyte diameter and the size of the central vein. Due to the AJ therapeutic effects, in all animals receiving MC+AJ, a significant decline was noticed on morphological changes, resembling a healthy organ. More differentiated microscopic features were also observed in the liver following MC administration like hyper-perfusion in the space of tissue, inflammatory cells aggregation (especially macrophages) around the CHV, CHV dilatation, and infiltration of lymphoid cells within portal space. Based on a histological rule, in any kind of organ inflammation or injury, the macrophages exocytose the chemical intermediates to regulate tissue injury.

In the liver, the Kupffer cells are available in the sinusoidal space of the liver which can be stimulated in response to tissue damages and secret Tumor Necrosis Factor-alpha, Interleukin-1, and NO leading to toxicity of the liver and necrosis of cells (Amouoghli Tabrizi, 2010). The MC is involved in extensive LP in hepatocytes. LP causes excessive cellular ROS and generation of radicals. ROS and free radical accumulation attack to unsaturated fatty acids in cell membranes, causing proteins alkylation (Almeer et al., 2020). ROS in elevated non-physiological levels changes the three-dimension structure of the active site in hepatic enzymes and causes incomplete cell function, which eventually leads to necrosis. Necrosis triggers hepatic inflammatory responses that are followed by penetration of mononuclear inflammatory cells. Simultaneously, the cells that are died by the pathological process of necrosis release inflammatory chemical intermediates leading to hepatitis (Salahshoor et al., 2019). Our data are in agreement with the findings by Zhou et al. (2020), which confirmed these pathological changes following MC administration. They stated that MC in an acute process could actively induce necrosis, blood hyperperfusion, and an increase in the Tumor Necrosis Factor-alpha expression rate. MC is able to destroy the mitochondrial membrane in an indirect cellular pathway causing a flux of mitochondrial enzymes into the cytoplasm and decreased total antioxidant activity (Jalili et al., 2019a, b). In oxidative stress onset and increased ROS/RNS condition, the mitochondrion is a primary damaged intracellular organelle (Salahshoor et al., 2018).

In this regard, it was documented that AJ can moderate LP process in cell cytosol and organelle membranes, and is also able to increase total antioxidant capacity in hepatocytes (indicating a reduction in levels of oxidative stress) (Sohrabinezhad et al., 2019). Thus, it is concluded that AJ, due to its antioxidant features, can moderate the level of LP into the normal state and increase total antioxidant potential level via inhibition of ROS production. As stated previously, based on histological assessments, the increased diameter of CHV is directly related to the cell necrosis after MC administration (Jalili et al., 2019a, b). AJ by antioxidant property can inhibit glutathione reduction. In conclusion, the activation of antioxidant enzymes following AJ administration enables the cells to cope with fatal effects of oxidative stress (Sohrabinezhad et al., 2019). Thus, it can be concluded that AJ as an exogenous agent regulates inflammatory pathways and inhibits apoptosis process (Kerishchi Khiabani and Bidaran, 2020). Kalantari et al. (2018a-c) in an experimental study, based on histological assessments stated that AJ has anti-cytotoxicity effects against oxidative stress, which manifests these therapeutic effects on hepatocytes. The results of this study support the concepts of Mumtaz et al. (2019), which explain that the liver damages and apoptosis initiation in hepatocytes can be produced via MC. Kalantari et al. (2018b) indicated that AJ reduces the MDA levels in the case of diazinon-induced toxicity in male Wistar rats, which is in agreement with our findings.

In this study, the results revealed a significant increase in liver enzyme activity in MC group. Furthermore, in whole members of AJ + MC group, a reduction trend was shown in the liver enzyme levels in comparison with the MC group. Any disruption in the liver cell membrane causes the enzymes released into the blood-stream (Salahshoor et al., 2018). It seems that MC can make destruction on the cell membrane through the inhibition of 1-4 respiratory chain complexes (Ben-Ozer et al., 2000). The results are in line with the conclusions by Emanuelli et al. (1996), which show that the MC in rats for 7 days of consumption induces a diminished level of TAC and equally amplified action of liver enzymes. It seems that AJ administration prevents enzymatic leakage and LP leading to stability of cellular membranes (Kerishchi Khiabani and Bidaran, 2019). Kalantari et al. (2018a) in an experimental investigation showed that AJ with the membrane-stabilizing property could lead to a reduction of serum markers of damaged liver, which is in line with the consequences of the current investigation. AJ by endoplasmic reticulum recruitment can cause cellular defense and anti-apoptosis properties (Candra et al., 2002).

The results of this study revealed that MC has potential to increase the serum level of nitrite oxide. Also, due to the beneficial effects of AJ, the serum level of nitrite oxide was reduced in compa-

rison with MC group confirming two main features of AJ on cells; the anti-inflammatory and antioxidant properties. In the mammalian body, the nitrite oxide is known as a free radical, thus interfering with the physiological manners at abnormal levels (Salahshoor et al., 2019). Along with nitrite oxide, the hydroxyl radical's superoxide anion can also cause hepatotoxicity (Jalili et al., 2019a). It has been proven that low-expression level of iNOS can significantly reduce the nitrite oxide production in cells. Antioxidants disrupt the molecular process of enzyme activity involved in nitrite oxide production (substrates, cofactors, and protein enzymes) (Wang et al., 2020). The results of the study by Sohrabinezhad et al. (2019) are in line with the results of this study, suggesting that AJ causes the expression of HO-1 and Calmodulin-calcium-dependent kinase-4 protein leading to NO-inducing Lipopolysaccharide inhibition.

Moreover, our information displayed a significant rise in the rate of inflammatory indicators expression in MC group. The relationship among the NO level and inflammatory factors has been revealed through lipopolysaccharide-induced lung damage in experimental animals (Somasundaram et al., 2020). Pro-inflammatory cytokines declined by AJ administration in bone marrow, but there was minor activity on TNFα and greater on IL6 production (Naeini et al., 2020). Consequently, we suggest a common positive connection between the level of NO, TLRs and inflammatory cytokines. However, in the inflammation process, we should not abandon the crucial role of the cell types in the intercellular interchange among definite TLR and pro-inflammatory cytokine. Similarly, in the case of MC -induced liver inflammation, we displayed a high positive correlation between NO and the expression of TNFα, IL-1β and, TLR4 while AJ reduces this unstable position. This outcome is aggravated when a study result by Jalili and colleagues was considered, defining the collaboration among TNFα, TLR-4 and IL-6 in protection cascade induced by Acacetin through Antioxidants Regulation in hepatitis subsequent ischemia-reperfusion (Jalili et al., 2019b). The current study is exclusive in characterizing either the relation of IL-1β and TLR-4 or this dependence in the case of MC administration and AJ inflammation salvage.

CONCLUSION

The present study showed that hepatoprotective properties of the hydro-alcoholic extract of AJ in contrary to MC-induced liver destruction in rats were observed. It was found that AJ reduces ROS, inflammatory cytokines, cell apoptosis, and expression of p53 and Bax genes, activation of antioxidant agents, and detoxification enzymes. Thus, AJ might be considered to recover the functional and histological characteristics of hepatocyte exposed to MC. This defending effect can be mediated through the antioxidant properties of AJ extract. AJ can be considered as a substitute beneficial agent against oxidative damages induced by toxic materials.

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