Original Article

Hepatoprotective Activity of Royal Jelly on Mercuric Chloride–induced Damage Model in Rats

Cyrus Jalili,
Shiva Roshankhah[1],
Amir Jalali[2],
Mohammad Reza Salahshoor[1]

Department of Anatomical Sciences, Medical Biology Research Center, [1]Department of Anatomical Sciences, Faculty of Medicine, [2]Department of Nursing, Faculty of Nursing and Midwifery, Kermanshah University of Medical Sciences, Kermanshah, Iran

Abstract

Background: Mercuric chloride (MC) is the chemical composition of mercury and chlorine with many side effects such as oxidative stress referring to mercury toxicity. Royal jelly (RJ) as a honey bee secretion has antioxidant activities. This study was designed to evaluate the effects of RJ against the parameters of hepatic damage in male rats induced by MC toxicity. **Materials and Methods:** In this study, 48 male rats were randomly assigned into six groups: sham (saline) and MC control (50 mg/kg) groups; RJ groups (200 mg/kg RJ for 1 day and 200 mg/kg RJ for 7 days, orally) and MC + RJ groups (200 mg/kg RJ orally + 50 mg/kg MC intraperitoneally for 1 day, and 200 mg/kg RJ orally and 50 mg/kg MC intraperitoneally for 7 days). Griess technique was used for the determination of serum nitric oxide (NO) level. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) concentrations were determined for liver functional disturbances value. In addition, thiobarbituric acid reactive species, antioxidant capacity, the diameter of hepatocytes, and the central hepatic vein (CHV) were investigated. **Results:** MC administration significantly increased the liver malondialdehyde (MDA) and NO levels, the mean diameter of CHV and hepatocyte, hepatic enzymes, and decreased tissue Ferric reducing antioxidant power (FRAP) level compared to the sham group ($P < 0.01$). The RJ and RJ + MC in all treatments significantly reduced the mean diameter of hepatocyte and CHV, hepatic enzymes, renal MDA and NO levels, and increased tissue FRAP level compared to the MC control group ($P < 0.01$). It seems that RJ administration recovers the hepatic injury induced by MC in rats.

Keywords: *Honey bees, liver/drug effects, mercuric chloride/toxicity*

Introduction

Increased production of reactive oxygen species (ROS) induces the secretion of inflammatory cytokines and lipid peroxidation and elevates the production of nitric oxide (NO) in different tissues.[1] Lipid peroxidation is an important factor involved in inducing oxidative damage in the cell membrane, lipoproteins, and other lipid structures.[2] Lipid peroxidation leads to the generation of peroxides and hydroxides, which causes toxicity in the body and a decline in antioxidant enzymes activity.[3] Mercuric chloride (MC) is the chemical composition of mercury and chlorine with the formula of $HgCl_2$.[4] It is widely used in analytical chemistry laboratories. This substance is transparent white or yellowish in color, acts as a part of chemical reference electrodes, and has been used for the treatment of syphilis. However, it is not used anymore due to mercury toxicity.[5] Rao and Purohit[6] showed that administration of MC significantly reduced the body weight, superoxide dismutase, catalase, and glutathione, and increased the lipid peroxidation. MC impairs the cells, cell membrane, and deoxyribonucleic acid (DNA), which leads to cell necrosis.[7] Some factors such as the increased level of ROS production, inflammatory cytokines, and NO are believed to be involved in MC toxicity in different tissues.[8] Administration of MC causes oxidative stress, which is followed by the production of ROS such as superoxide radical (H_2O_2) and hydroxyl radical (OH), induction of cell damage by biomembrane lipids destruction, and disruption of cellular metabolism.[9] The oxidative stress induced by lipid peroxidase production causes the hepatic cell membrane damage and triggers the activity of some hepatic enzymes.[10] Presentation of antioxidant compounds can be an appropriate strategy to decrease the oxidative stress-induced damage.[11] Royal jelly (RJ) plays key roles in neonatal growth, completion of special sexual features, and long life of the queen. Hypopharyngeal glands of honeybees by the production of RJ play a crucial role in beehive development, especially queen.[12] The bees feed RJ in the first 3 days

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

How to cite this article: Jalili C, Roshankhah S, Jalili A, Salahshoor MR. Hepatoprotective activity of royal jelly on mercuric chloride–induced damage model in rats. J Rep Pharm Sci 2019;8:181-7.

Address for correspondence:
Dr. Mohammad Reza Salahshoor, Department of Anatomical Sciences, Faculty of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran.
E-mail: reza.salahshoor@yahoo.com

Access this article online
Website: www.jrpsjournal.com
DOI:10.4103/jrptps.JRPTPS_27_19
Quick Response Code:

and their diet is changed afterward, whereas the queen always feeds RJ.[13] RJ contains a wide range of chemical compounds such as proteins (17%–45% dry weight), royalism (with potent antibacterial properties), apisimin (stimulating the proliferation of monocytes in human), Jelleines (with antimicrobial activity), lipids, fatty acids, carbohydrates, and vitamins (B group vitamins, thiamine, riboflavin, pentatonic acid, niacin, folic acid, and biotin).[14] The antioxidant activity of RJ inhibits the effects of oxidative stress and lipid peroxidation and protects DNA against oxidative stress.[15] Kanbur *et al.*[16] reported that mice fed with RJ had a higher level of 8-hydroxy-2-decenoic acid (oxidative stress marker) in their liver and blood serum, with increased average longevity. Further, Karadeniz *et al.*[17] showed that the administration of RJ decreased liver toxicity and oxidative stress induced by cisplatin compounds. MC has toxic effects and RJ has numerous beneficial properties, especially antioxidant features. Further, no study has ever investigated the effects of RJ on the MC-induced disorders of the liver. Hence, this study was carried out to explore the effects of RJ on MC-induced impairments of the liver of male rats.

Materials and Methods

Chemicals and kits

MC is the chemical composition of mercury and chlorine with the formula of $HgCl_2$. $HgCl_2$, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT) kits, and all other necessary reagents of analytical grade were bought from Sigma-Aldrich (St Louis, Missouri). In addition, 20 g of the RJ vial (Pars Asal, Shiraz, Iran) was prepared in 1 L of sterilized double distilled water in accordance with the instructions of the manufacturer.

Animals

Animals were treated according to the guidelines of animal care and handling developed by the Iran's Ministry of Health. Forty-eight male Wistar rats (weighing 220–250 g) were purchased from Pastor Institute of Iran. The animals were housed in standard cages (three per cage) with control conditions at 23°C ± 2°C and exposed to 12-h light/dark cycle in animal house of school of the medicine. The animals were exposed to care facilities including water and food a week before treatment. All investigations conformed to the ethical and humane principles of research and were approved by the Ethics Committee of Medical Sciences (ethics certificate no. 1397.945).[11]

Groups and treatment

Forty-eight male rats were randomly divided into six groups (eight rats in each group). First group, the sham group that received normal saline (through intraperitoneal [IP] injection) equivalent to the amount of experimental groups. Second group, the MC control group (a single dose of 50 mg/kg of MC IP injection). Third to fourth groups, the RJ administration groups (oral administration of 200 mg/kg RJ for 1 day and 200 mg/kg for 7 days at 10 AM). Fifth to sixth groups, MC + RJ administration groups (200 mg/kg RJ + 50 mg/kg MC for 1 day and 200 mg/kg RJ + 50 mg/kg MC for 7 day at 10 AM).[4,16]

Dissection and sampling

Rats related to each group were anesthetized with ether inhalation, 24 h after the last injection. Venipuncture of the animals' hearts (right ventricle) was carried out using a 5-cc syringe. The blood sample was incubated to make a clot for 15 min at 37°C. Then the clot centrifuged to separate the serum at 3000 rpm for 15 min. The serum was stored at −70°C for the measurement of some biochemical parameters of the liver, NO level, oxidative stress, and antioxidant capacity level. Then, the animals' liver was fixed in 10% formalin solution for histological and morphometric investigations.[1]

Oxidative stress

To assess the oxidative stress by colorimetric analysis, the thiobarbituric acid (TBA) reactive species were measured using malondialdehyde (MDA) as the final product of lipid peroxidation of liver. In brief, three separate solutions each with a volume of 1400 μL including acetic acid (Sigma-Aldrich), TBA (Sigma-Aldrich), and sodium dodecyl sulfate (Sigma-Aldrich) were added to 100 μL of liver homogenate, and the mixture was stirred for 50 min. A total of 4 mL of 1-butanol (Sigma-Aldrich) was added to the combination and vortexed by centrifugation at 5000 rpm for 15 min. The absorbance of the higher layer was measured at 532 nm on spectrometer (Spectro; Germany) and sequential concentrations of tetraethoxypropane (Sigma-Aldrich) were used as the external standard. The antioxidant capacity of the liver was measured using Ferric reducing antioxidant power (FRAP) assay. The FRAP substance consisted of 30 mL of acetate buffer (Sigma-Aldrich) and 1.5 mL chloride ferric (Sigma-Aldrich). Briefly, 60 μL of kidney homogenate was added to 1.5 mL of newly prepared FRAP substance (Sigma-Aldrich) available in a test tube incubated at 37°C for 10 min. The absorbance of the blue-colored complex was read against a blank at 593 nm. Sequential concentrations of $FeSO_4.7H_2O$ (Sigma-Aldrich) were used as an external standard.[11]

Enzymes

The liver was split and turned into a homogenous solution. To separate the biological enzymes, the obtained solution was centrifuged twice at 10,000 rpm for 15 min. The supernatant was separated for enzyme measurement. ALT and AST activities were examined by the Reitman and Frankel method. ALP activities were determined according to the procedure set out in the practical laboratory manual.[2]

Nitric oxide assay

NO measured by Griess assay according to the microplate technique. To measure the concentration of serum nitrite, the sample was defrosted and the supernatant (400 μL) was deproteinized by zinc sulfate under the condition of centrifugation (6 mg zinc sulfate powder was mixed with 400 μL serum and vortexed for 1 min). A total of 100 μL vanadium chloride, 50 μL *N*-(1-naphthyl) ethylenediamine dihydrochloride, and 50 μL sulfonamide solutions were added to the 100 μL of the supernatant. Sodium nitrite (0.1 M) was used for the standard curve, and increasing concentrations of

sodium nitrite (5, 10, 25, 50, 75, and 100 µM) were prepared. The Griess solution was added to all microplates containing sodium nitrite and supernatant, and finally the reading was obtained through an enzyme-linked immunosorbent assay (ELISA) reader (Stat Fax 100, Miami, FL, USA) at the wavelength of 540 nm.[10]

Morphological and histopathological examinations

For histological evaluation of the hepatic structures, a piece of liver just 1 cm below the right lobe was removed in transverse section, washed in normal saline, and fixed in 10% formalin. The sample was dehydrated in ascending concentrations of ethanol, cleared by xylene solution, and then embedded in paraffin. Thin sections (4 µm) were cut using a microtome (Leica RM 2125, Nussloch, Germany) and stained with hematoxylin and eosin. For each hepatocyte, the full cellular area was measured. The hepatocyte outline was measured after capturing an image with a 40× objective lens. The maximum and minimum axis was measured for each hepatocyte to obtain the mean axis. At least 50 hepatocytes from each zone were measured in each liver. A separate measurement of central hepatic vein (CHV) was performed using the same assay. The planning was examined with an Olympus)BX-51T-32E01 (research microscope connected to a DP12 Camera with a 3.34-million pixel resolution and Olysia Bio-software (Olympus Optical, Tokyo, Japan).[3]

Statistical analysis

After extracting the data, the Kolmogorov–Smirnov test was conducted first to confirm the data compliance of the normal distribution. One-way analysis of variance (one-way ANOVA) was used for statistical analysis and Tukey *post hoc* test was used to determine the differences among the groups. The Statistical Package for the Social Sciences (SPSS, New York: IBM, SPSS version 20.0) software, version 16, was used for data analysis, and the results were expressed as mean ± standard error, and $P < 0.05$ was considered significant.

Results

Oxidative stress

The results of the oxidative stress testing showed that the liver MDA level significantly increased in the MC control group compared to that in the sham group ($P < 0.01$). The liver MDA level decreased significantly in all MC + RJ groups compared to that in the MC control group ($P < 0.01$). Similarly, the MC significantly decreased the liver FRAP level of the MC control group in comparison with that of the sham group ($P < 0.01$). Administration of RJ significantly increased the FRAP level in the liver in all MC + RJ groups compared to that in the MC control group ($P < 0.01$). Treatment with RJ in all groups had no significant difference in the liver FRAP and liver MDA levels compared to that in the sham group ($P > 0.05$) [Figure 1].

Liver enzymes

MC led to a significantly increased ALT, AST, and ALP enzymes in comparison with the sham group ($P < 0.01$). The mean concentration of ALT, AST, and ALP enzymes showed no significant difference in all RJ groups compared to that in the sham group ($P > 0.05$). Also, in all RJ and MC + RJ groups, significant decrease was recorded

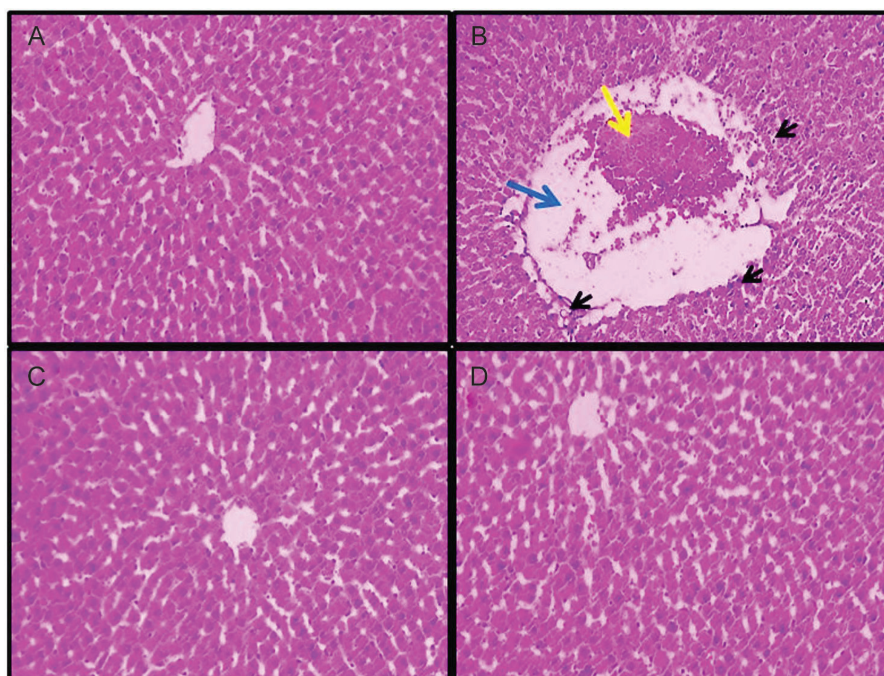

Figure 1: Microscopic images of liver of mature rats in different groups (5 µm, hematoxylin and eosin staining, ×100). Micrograph of the liver section in the sham group (A), normal liver structure. Micrograph of the liver section in mercuric chloride (MC) control group (B), increased white blood and macrophage cells (inflammation) (black arrows), central hepatic vein dilatation (blue arrow), and hyperemia (yellow arrow) due to the oxidative stress caused by MC. Micrograph of the liver section in royal jelly (RJ) (200 mg/kg/7 day) group (C), normal liver structure. Micrograph of liver section in RJ +MC (200 mg/kg/7 day) group (D), normal liver structure

in the mean value of ALT, AST, and ALP enzymes in comparison with the MC control group ($P < 0.01$) [Table 1].

Nitric oxide

The results of blood serum NO level measurement showed a significant increase in MC control group compared to that in the sham group ($P < 0.01$). The mean NO level in the blood serum showed no significant difference in all RJ groups compared to that in the sham group ($P > 0.05$). Also, the mean of NO level in blood serum significantly reduced in all RJ and MC + RJ groups compared to that in the MC control group ($P < 0.01$) [Figure 2].

Morphometric measurements

The mean diameter of hepatocytes and CHV in experimental groups showed a significant increase between the sham group and the MC control group ($P < 0.01$). The mean diameter of hepatocytes and CHV showed no significant difference in all RJ groups compared to that in the sham group ($P > 0.05$). Further, RJ and MC + RJ significantly reduced the mean diameter of hepatocytes and CHV in all treated groups in comparison with the MC control group ($P < 0.01$) [Figure 3].

Histopathological changes

Histological analysis showed normal renal structure in the sham and RJ treatment group. After treatment with MC in MC control group, the liver showed obvious changes and injuries. These anomalies included the increment in white blood cells (inflammation), increased irregularities, sinusoidal dilatation, and the vacuolization hepatocyte (necrosis). Treatment with MC + RJ in all treated groups reduced the renal damage caused by the MC toxicity [Figure 4].

Discussion

MC with high mercury content can induce a wide range of damages such as disruption in the development of central nervous system, impairment of retina cells, hepatic and cardiovascular diseases, autoimmune disorders, and allergy.[18] The findings of the current research suggest that MC administration has adverse and destructive effects on liver histology and function, oxidant–antioxidant imbalance, and increase in NO level. The RJ as a natural antioxidant, decrease the side effects of MC administration on the liver. It also recovers the cell damage by reduced MDA level, histology alterations, and the rate of oxidation. This study results also show that RJ is able to reduce the lipid peroxidation (decreased MDA) and to increase the antioxidant capacity of liver causing a reduction in oxidative stress. Consistent with these findings, a large body of studies has shown the antioxidant properties of RJ.[15-17] RJ seems to inhibit the lipid peroxidation induced by tert-butyl

Table 1: Different hepatic enzymes between treatment groups

Enzymes (ng/mL)	Sham	MC control (50 mg/kg)	RJ (200 mg/kg) 1 day	RJ (200 mg/kg) 7 days	RJ (200 mg/kg) + MC (50 mg/kg) 1 day	RJ (200 mg/kg) + MC (50 mg/kg) 7 days
AST	76.93 ± 2.20	121.8 ± 5.10*	76.33 ± 3.40**	74 ± 2.010**	95.4 ± 5.20***	90.8 ± 6.00***
ALT	35.49 ± 2.50	59.14 ± 4.10*	34.91 ± 2.50**	33.7 ± 1.10**	38.7 ± 5.01***	38.8 ± 2.60***
ALP	2.14 ± 0.10	5.28 ± 0.20*	2.05 ± 0.30**	2.1 ± 0.50**	3.05 ± 0.30***	3.0 ± 0.10***

MC = mercuric chloride, RJ = royal jelly, ALP = alkaline phosphatase, ALT = alanine aminotransferase, AST = aspartate aminotransferase
*$P < 0.001$ compared to the sham group, **$P < 0.001$ compared to MC control group, ***$P < 0.001$ compared to the MC control group
Data were presented as mean ± standard deviation

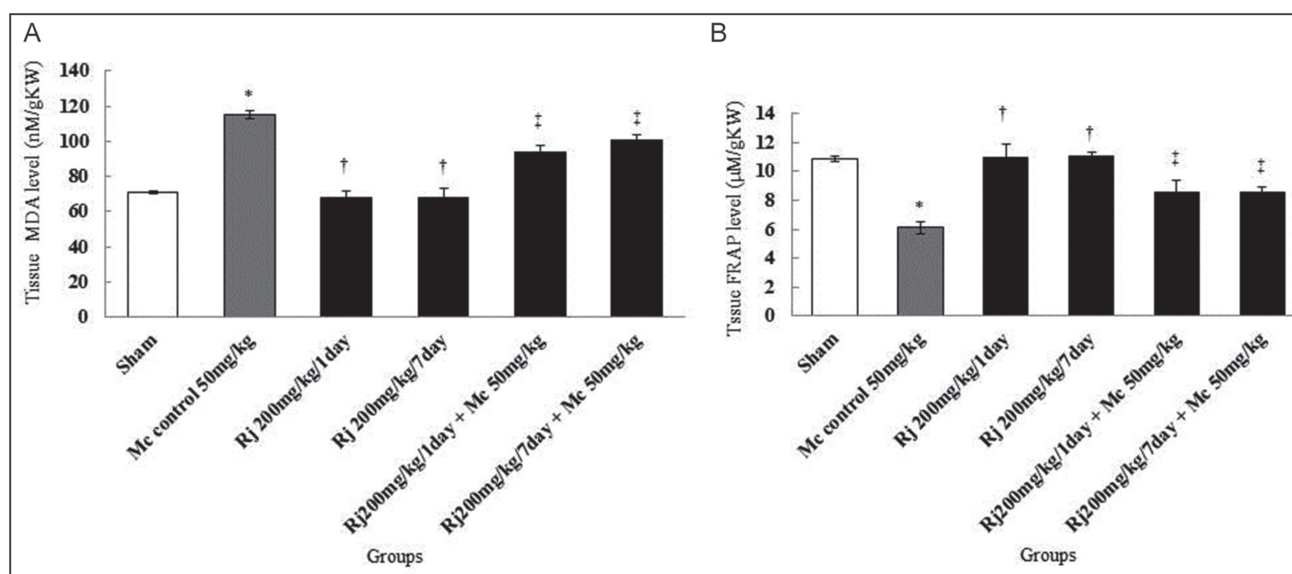

Figure 2: Comparison of mercuric chloride (MC), sham, and royal jelly (RJ) groups of: (A) hepatic malondialdehyde (MDA) level and (B) tissue FRAP level. *Significant deferent compared to the sham group ($P < 0.01$). †Significant deferent compared to the MC control group ($P < 0.01$). ‡Significant deferent compared to the MC control group ($P < 0.01$).

hydroperoxide in the liver.[19] Further, RJ is a lipophilic molecule that is able to inhibit the lipid peroxidation via Fenton reaction.[16] Hesami *et al.*[20] showed that RJ attenuated the oxidative stress, blood glucose, and lipid peroxidation in patients with type II diabetes, which is in line with the results of this study. Thus, it appears that RJ with its antioxidant properties could reduce the MDA in the treatment groups by inhibiting the production of ROS. This study also indicates the recovery effects of RJ on some liver parameters as well as decreasing the oxidative stress because of the decline in MDA. The toxicity of MC administration can lead to blood, biochemical, and oxidative stress changes. Therefore, the mechanism of MC compound toxicity is implemented by oxidative stress.[4] The findings revealed that the number of hepatocytes in the control group of MC has significantly decreased compared to that in the sham group, whereas the CHV size has significantly increased. In addition, there was a significant increase in the number of hepatocytes and a significant decrease in the size of the CHV in all RJ and MC + RJ groups compared to that in the control MC administration group. Another important finding revealed some changes observed in the liver in the control group of MC administration, including the enhanced state of the sinus spaces, macrophages accumulation around the CHV, and the lymphoid cells penetrated in the portal space, as well as the CHV diameter enlargement. It seems that the invasion of free radicals to hepatic cells causes necrosis in cells of hepatic parenchyma.[1] These cells can induce inflammatory responses in the liver, which leads to tissue damage by mononuclear inflammatory cells. The necrotic cells release pro-inflammatory mediators, which can exacerbate the poison-induced liver injury.[3] Apparently, macrophages are activated in response to tissue injury and release positive mediators, such as the alpha tumor necrosis factor, interleukin-1, and NO.[10] In this study, the macrophages are actually the same as Kupffer cells located in the hepatic sinuses. It may seem that the Kupffer cell accumulation and the secretion of toxic mediators in these areas, with no symptoms of cell death, are involved in the presence of liver toxicity and necrosis.[2] Moreover, production of free radicals and subsequent oxidative stress can be one of the most critical and essential causes of the liver cells death.[1] The results corroborate the ideas of Mumtaz *et al.*,[21] which suggested that the hepatic injuries and apoptosis induction in hepatocytes can be caused by MC. MC administration, induced of free radical production, may invade to hepatic cells and cause necrosis in parenchymal cells. These cells can trigger the inflammatory responses in the liver and cause the invasion of mononuclear inflammatory cells to damaged tissue. The necrotic cells release

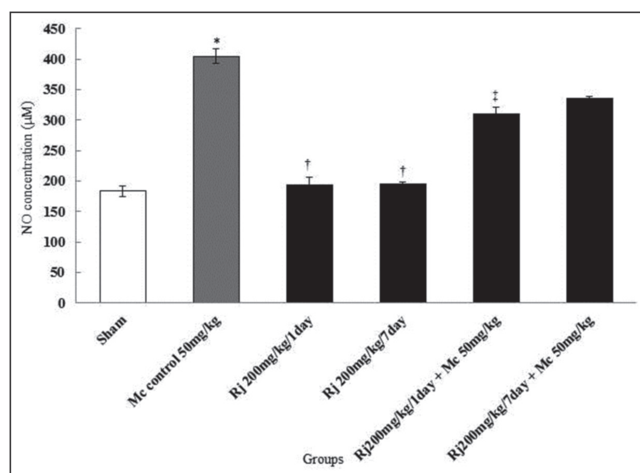

Figure 3: Effects of royal jelly (RJ), mercuric chloride (MC), and RJ + MC on the mean nitric oxide (NO) levels. *Significant increase compared to the sham group (*P* < 0.01). †Significant decrease compared to the MC control group (*P* < 0.01). ‡Significantdecrease compared to the MC control group (*P* < 0.01)

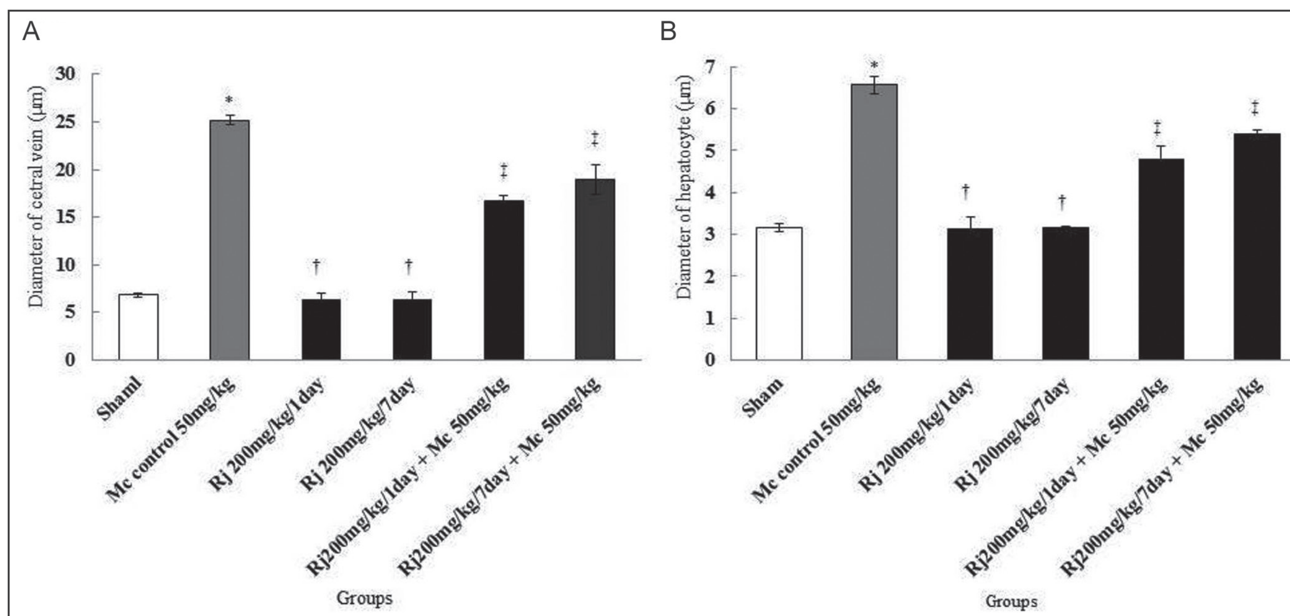

Figure 4: Effects of mercuric chloride (MC), royal jelly (RJ), and RJ + MC administration on the central hepatic vein (A) and diameter of hepatocyte (B). *Significant increase compared to the sham group (*P* < 0.01). †Significant decrease compared to the MC control groups (*P* < 0.01). ‡Significant decrease compared to the MC control group (*P* < 0.01)

the pro-inflammatory mediators, which can exacerbate toxin-induced hepatic injuries.[10] It may seem that the oxidative stress induced by MC administration can produce active ROS with notable examples as hydroperoxides, singlet oxygen, hydrogen peroxide, and superoxide that lead to the destruction of cell, DNA, proteins, and intracellular lipids, and ultimately leads to hepatic injury.[22] RJ appears to carry out a protective effect against hepatic fibrogenesis. This effect is due to the following features such as the polyphenol capacity, inhibitory effect on stellate cells' activity, disorganization of the signal transduction pathways, and expression of the cell cycle proteins.[23] Stellate cells play a crucial role in the improvement of hepatic fibrosis and oxidative stress.[2] It seems that RJ with its capability can inhibit the P38MAPK phosphorylation in activated lipopolysaccharide of microglia.[24] RJ can exert its anti-inflammatory effects on nuclear factor kappa B pathway. RJ can inhibit the NF-Kβ by reducing H_2O_2 production, inhibiting the inhibitor kappa β kinase, phosphorylation of P65, and depletion of P65.[25] Silici *et al.*[26] showed that RJ inhibits the induction of cisplatin-induced nephrotoxicity through the inhibition of oxidative stress, which is in line with the findings of this study. The results of this study indicate that there are significant differences between liver antioxidant capacity and AST, ALT, and ALP levels in the control MC group and the sham group. Similarly, there is a negative correlation in all treatments between hepatic antioxidant capacity in the control MC group and AST, ALT, and ALP levels in the group that received RJ and MC + RJ. The increase in the activity index of hepatic enzymes that exist in serum indicates the liver injury in this study. Moreover, the findings of Kanbur *et al.* confirmed the results of this study in that RJ could decrease the serum levels of ALT, AST, and ALP.[16] These enzymes can be released into the blood flow due to the incidence of necrosis or cell membrane damage.[1] It may seem that MC can induce damage to the cell membrane integrity by the inhibition of 1–4 respiratory chain complexes.[27] The results are in agreement with the findings of a study by Emanuelli *et al*., which revealed that the MC administration in male rats for 1 week induces increased activity of liver enzymes and conversely reduced the total antioxidant capacity.[28] RJ appears to stabilize the cell membranes and prevents the leakage of enzymes by lipid peroxidation prevention.[15] RJ can exert its antioxidant and anti-inflammatory effects by inducing antioxidant enzymes, adjusting lipid metabolism and reducing lipid peroxidation.[16] The results are consistent with the studies suggesting that administration of RJ reduces hepatic enzymes in diabetic rats and prevents hepatocytes injuries.[29] The results of this study indicate a significant increase in the amount of NO in the serum of the recipient control MC group compared to that in the sham group. Furthermore, a significant decrease of serum NO level was observed in MC + RJ group compared to that in control MC group. It seems that oxidative stress available in cells increases the synthesis of NO synthase, and consequently leads to an increase in nitrite production and decrease in cell survival.[11] Because of the high consumption of oxygen, the mitochondrial dysfunction may increase the production of free radicals such as NO in most tissues of the body, and also it may induce tissue injury, especially in the liver because of the oxidative and nitrosative stress.[1] Administration of MC can significantly increase the amount of nitrotyrosine and NO biomarkers in the liver through the induction of oxidative stress.[30] On the contrary, the antioxidants can damage and degrade the NO system (protein enzymes, substrates, and cofactors), hence can reduce its production.[2] The results are in agreement with the findings of a study by Sugiyama *et al.*, which showed that RJ can reduce NO in morphine-induced damage of liver.[31] The results are in accordance with recent studies indicating that MC by the means of oxidative stress induction is apparently able to damage and degrade hepatocytes, reduce antioxidant capacity, and elevate serum levels of hepatic enzymes and NO. On the contrary, RJ with a potent antioxidant property can reduce the detrimental effects of these destructive substances to some extent. Accordingly, the application of RJ can be a proper strategy to reduce the levels of free radicals and prevent the hepatic injuries of people who are exposed to mercuric drugs, specifically patients.

Conclusion

It appears that RJ may recover some hepatic damages in rats treated with MC. Hence, it could be considered to improve the histological and functional features of the liver exposed to MC. The study approves the eliminated hepatic oxidant–antioxidant balance as molecular advocator because the administration of MC would supervise the cellular chain reaction, observable either with light microscopy. However, supplementary studies are necessary to accurately describe the mechanisms of the action. RJ activates the oxidant system following MC administration.

Acknowledgement

We gratefully acknowledge the Research Council of Kermanshah University of Medical Sciences (No: 97945) for the financial support.

Financial support and sponsorship

This study supported by the Research Council of Kermanshah University of Medical Sciences, Kermanshah, Iran.

Conflicts of interest

There are no conflicts of interest.

References

1. Salahshoor MR, Mohammadi MM, Roshankhah SH, Najari N, Jalili C. Effect of *Falcaria vulgaris* on oxidative damage of liver in diabetic rats. J Diabetes Metab Disord 2019;19:1-9.
2. Zhu Z, Zhang Y, Wang J, Li X, Wang W, Huang Z. Characterization of sugar composition in Chinese royal jelly by ion chromatography with pulsed amperometric detection. J Food Compos Anal 2019;78:101-7.
3. Salahshoor MR, Roshankhah S, Hosseni P, Jalili C. Genistein improves liver damage in male mice exposed to morphine. Chin Med J (Engl) 2018;131:1598-604.

4. Ansar S, Iqbal M. Protective effect of diallylsulphide against mercuric chloride-induced hepatic injury in rats. Hum Exp Toxicol 2016;35:1305-11.
5. Koli S, Prakash A, Choudhury S, Mandil R, Garg SK. Calcium channels, rho-kinase, protein kinase-C, and phospholipase-C pathways mediate mercury chloride-induced myometrial contractions in rats. Biol Trace Elem Res 2019;187:418-24.
6. Rao MV, Purohit AR. Neuroprotection by melatonin on mercury induced toxicity in the rat brain. Pharm Pharm 2011;2:375-85.
7. Betti C, Barale R, Pool-Zobel BL. Comparative studies on cytotoxic and genotoxic effects of two organic mercury compounds in lymphocytes and gastric mucosa cells of Sprague-Dawley rats. Environ Mol Mutagen 1993;22:172-80.
8. Ahmad S, Mahmood R. Mercury chloride toxicity in human erythrocytes: enhanced generation of ROS and RNS, hemoglobin oxidation, impaired antioxidant power, and inhibition of plasma membrane redox system. Environ Sci Pollut Res 2019;5:1-3.
9. Liu B, Yu H, Baiyun R, Lu J, Li S, Bing Q, *et al*. Protective effects of dietary luteolin against mercuric chloride-induced lung injury in mice: Involvement of AKT/nrf2 and NF-κb pathways. Food Chem Toxicol 2018;113:296-302.
10. Salahshoor MR, Khashiadeh M, Roshankhah S, Kakabaraei S, Jalili C. Protective effect of crocin on liver toxicity induced by morphine. Res Pharm Sci 2016;11:120-9.
11. Jalili C, Moradi D, Roshankhah S, Salahshoor MR. Effect of pentoxifylline on kidney damage induced by nitrosamine in male rats. Res Pharm Sci 2019;14:64-73.
12. Dalfardi M, Taghavi MM, Shariati Kohbanani M, Taghipour Z, Nosratabadi R, Jalili C, *et al*. Protective and modulatory effects of royal jelly used against the induced changes in silver nanoparticles on the hippocampus of male rats. Nanomed J 2019;6:136-41.
13. Hossen MS, Nahar T, Gan SH, Khalil M. Bioinformatics and therapeutic insights on proteins in royal jelly. Curr Proteomics 2019;16:84-101.
14. Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Álvarez JA. Functional properties of honey, propolis, and royal jelly. J Food Sci 2008;73:R117-24.
15. Maqsoudlou A, Mahoonak AS, Mora L, Mohebodini H, Toldrá F, Ghorbani M. Peptide identification in alcalase hydrolysated pollen and comparison of its bioactivity with royal jelly. Food Res Int 2019;116:905-15.
16. Kanbur M, Eraslan G, Beyaz L, Silici S, Liman BC, Altinordulu S, *et al*. The effects of royal jelly on liver damage induced by paracetamol in mice. Exp Toxicol Pathol 2009;61:123-32.
17. Karadeniz A, Simsek N, Karakus E, Yildirim S, Kara A, Can I, *et al*. Royal jelly modulates oxidative stress and apoptosis in liver and kidneys of rats treated with cisplatin. Oxid Med Cell Longev 2011;2011:981793.
18. Houston MC. The role of mercury and cadmium heavy metals in vascular disease, hypertension, coronary heart disease, and myocardial infarction. Altern Ther Health Med 2007;13:S128-33.
19. Almeer RS, Kassab RB, AlBasher GI, Alarifi S, Alkahtani S, Ali D, *et al*. Royal jelly mitigates cadmium-induced neuronal damage in mouse cortex. Mol Biol Rep 2018;9:1-3.
20. Hesami S, Hashemipour S, Shiri-Shahsavar MR, Koushan Y, Khadem Haghighian H. Administration of Iranian propolis attenuates oxidative stress and blood glucose in type II diabetic patients: a randomized, double-blind, placebo-controlled, clinical trial. Caspian J Intern Med 2019;10:48-54.
21. Mumtaz S, Ali S, Khan R, Andleeb S, Ulhaq M, Khan MA, *et al*. The protective role of ascorbic acid in the hepatotoxicity of cadmium and mercury in rabbits. Environ Sci Pollut Res 2019;9:1-10.
22. Elblehi SS, Hafez MH, El-Sayed YS. L-α-phosphatidylcholine attenuates mercury-induced hepato-renal damage through suppressing oxidative stress and inflammation. Environ Sci Pollut Res 2019;5:1-10.
23. Bilgic S, Dogan Z, Azirak S, Erdemli ME, Onderci M, Turk A, *et al*. Hepatoprotective effect of royal jelly, grape seed extract, and *Lycium barbarum* against diethylnitrosamine-induced liver toxicity in rats. J Turgut Ozal Med Cent 2018;25:342-8.
24. Yang XY, Yang DS, Wang JM, Li CY, Lei KF, Chen XF, *et al*. 10-Hydroxy-2-decenoic acid from royal jelly: A potential medicine for RA. J Ethnopharmacol 2010;128:314-21.
25. Dzopalic T, Vucevic D, Tomic S, Djokic J, Chinou I, Colic M. 3, 10-Dihydroxy-decanoic acid, isolated from royal jelly, stimulates Th1 polarising capability of human monocyte-derived dendritic cells. Food Chem 2011;126:1211-7.
26. Silici S, Ekmekcioglu O, Kanbur M, Deniz K. The protective effect of royal jelly against cisplatin-induced renal oxidative stress in rats. World J Urol 2011;29:127-32.
27. Ben-Ozer EY, Rosenspire AJ, McCabe MJ Jr, Worth RG, Kindzelskii AL, Warra NS, *et al*. Mercuric chloride damages cellular DNA by a non-apoptotic mechanism. Mutat Res Genet Toxicol Environ Mutagen 2000;470:19-27.
28. Emanuelli T, Rocha JB, Pereira ME, Porciuncula LO, Morsch VM, Martins AF, *et al*. Effect of mercuric chloride intoxication and dimercaprol treatment on delta-aminolevulinate dehydratase from brain, liver and kidney of adult mice. Pharmacol Toxicol 1996;79:136-43.
29. Ghanbari E, Nejati V, Khazaei M. Improvement in serum biochemical alterations and oxidative stress of liver and pancreas following use of royal jelly in streptozotocin-induced diabetic rats. Cell J 2016;18:362-70.
30. Sarwar Alam M, Kaur G, Jabbar Z, Javed K, Athar M. *Eruca sativa* seeds possess antioxidant activity and exert a protective effect on mercuric chloride induced renal toxicity. Food Chem Toxicol 2007;45:910-20.
31. Sugiyama T, Takahashi K, Kuzumaki A, Tokoro S, Neri P, Mori H. Inhibitory mechanism of 10-hydroxy-trans-2-decenoic acid (royal jelly acid) against lipopolysaccharide- and interferon-β-induced nitric oxide production. Inflammation 2013;36:372-8.