AJPR
Avicenna Journal of Pharmaceutical Research
Avicenna J Pharm Res 2023;4(2)55-61. doi:10.34172/ajpr.1089
http://ajpr.umsha.ac.ir
UMSHA Press

Original Article

Hepatoprotective Effects of Geraniol and Nanogeraniol Against Paraquat Toxicity in Male Rats

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Article history:
Received: July 5, 2023
Revised: ugust 18, 2023
Accepted: September 29, 2023
ePublished: November 23, 2023

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Abstract

Background: The hepatotoxicity of paraquat (PQ) is well known. Given geraniol's antioxidant activity, the present study compared the therapeutic effectiveness of geraniol and nanogeraniol (NG) against PQ-induced hepatotoxicity in male rats.

Methods: Thirty male Wistar rats were assigned to six separate groups. Hepatotoxicity was induced with PQ (5 mg/kg/day, p.o.), and two treatment groups received the same concentration of geraniol and NG (100 mg/kg/day, p.o.) for seven days. The emulsification-solvent evaporation method was applied to prepare NG. At the end of the treatment period, the animals were deeply anesthetized, and the serum and liver tissue samples were separated. Moreover, liver function enzymes, such as alanine and aspartate aminotransferase (ALT and AST), were assessed in the serum samples. Finally, oxidative stress (OS) biomarkers and histopathological studies were evaluated in the liver tissue samples.

Results: The results revealed that PQ administration could significantly increase the serum levels of ALT and AST. Additionally, PQ increased lipid peroxidation while decreasing the levels of the antioxidant system, indicating the induction of OS.

Conclusion: However, treatment with NG was potentially superior to free geraniol in returning the mentioned ratio to normal levels, and histopathological examination correlated well with these findings. Thus, NG, due to its antioxidant activity, can be used as a new and efficient therapeutic approach in treating hepatotoxicity caused by PQ compared to the free geraniol.

Keywords: Paraquat, Geraniol, Nanogeraniol, Liver, Oxidative stress

Please cite this article as follows: Akbari Moheb A, Firouzian F, Ahmadi Moghadam D, Mirzaei F, Ranjbar A. Hepatoprotective effects of geraniol and nanogeraniol against paraquat toxicity in male rats. Avicenna J Pharm Res 2023;4(2)55-61. doi:10.34172/ajpr.1089

Introduction

Paraquat (PQ) is a fast-acting and non-selective herbicide that is commonly used to remove weeds in agriculture (1). Nonetheless, PQ is well-known as a serious hazard to humans, and short-term and long-term exposure (e.g., inhalation and skin contact) to PQ can cause severe damage to various organs in humans and animals (2, 3). Although lung toxicity and pulmonary fibrosis are the initial consequences of PQ exposure (4), hepatotoxicity is one of the serious results of PQ poisoning (5). By interfering with the formation of reactive oxygen species (ROS), oxidative stress (OS) may be the potential toxic mechanism of PQ (6). Extensive oxygen anions and ROS are produced after PQ enters the cell and causes mitochondrial dysfunction. Therefore, increasing the contents of ROS leads to deleterious consequences, such as the demolition of unsaturated lipids in cell membranes, lipid peroxidation (LPO), and the impairment of the antioxidant defense system balance (7, 8).

The absence of an effective antidote for PQ poisoning results in a high mortality rate worldwide. In this regard, although some studies have demonstrated the protective effects of several agents with various possible mechanisms, antioxidant therapy has received much attention in healthcare facilities as a suitable option to reduce PQ-induced toxicities (9, 10).

Geraniol (3,7-dimethylocta-trans-2,6-dien-1-ol) is an acyclic monoterpene alcohol extracted from palmarosa, ninde, and rose essential oils (11). Considering that geraniol is associated with several pharmacological features, such as antitumor (12), antimicrobial (13), antioxidant (14), and anti-inflammatory (15) activities, through multiple signaling pathway regulation, geraniol can be a promising drug. Furthermore, some studies confirmed the hepatoprotective effects of geraniol based on decreased alanine and aspartate aminotransferase (ALT and AST) serum levels, improved mitochondrial functions by suppressing ROS, the inhibition of tumor necrosis factor-alpha and interleukin-6 expression, inhibition of myeloperoxidase activity, reduction of LPO, and enhanced antioxidant enzyme activities in a rat model (16, 17).

Drug delivery systems (DDS) are strongly thought to play a crucial role in improving the pharmacological and therapeutic properties of drugs (18). Nanostructured lipid carriers (NLCs) are among the most common types of lipid-based formulations to enhance the oral bioavailability of aqueous insoluble bioactive compounds without any adverse effects (19). NLCs have gained attention over other DDSs due to biocompatibility, biodegradability, increased drug bioavailability, physicochemical and colloidal stability, nano-size, improved drug loading capacity, and trapping efficacy (20).

Accordingly, the current study aims to compare the efficiency of geraniol-loaded NLCs compared to free geraniol against PQ-induced hepatotoxicity in male rats.

Materials and Methods

5,5'-dithio-bis-[2-nitrobenzoic acid] (DTNB), 2,4,6-Tripyridyl-s-Triazine (TPTZ), Tris base, and thiobarbituric acid utilized in this experiment were purchased from Sigma Chemical Company. In addition, geraniol was provided by Alfa Aesar, a Johnson Matthey Company (London, UK).

Preparation of Geraniol-Loaded Nanostructured Lipid Carriers

In this research, nanoparticles (NPs) were prepared by the emulsion-evaporation of the solvent with some modifications (20, 21). The aqueous phase was prepared by mixing 1500 mg of poloxamer 188 and heating it at 75°C on the stirrer for 7 minutes. Further, glyceryl monooleate (300 mg), oleic acid (300 mg), and geraniol (30 mg) were dissolved in 1 mL of ethanol, and then the transparent solution was obtained by heating the mixture. Next, the lipid phase was added to the aqueous phase dropwise in a hot water bath at 75°C under homogenization at 12,000 rpm using a Heidolph VR homogenizer (Schwabach, Germany) for 20 minutes. Afterward, the obtained emulsion was placed in an ice bath and sonicated for 15 minutes (on: 20 seconds, off: 3 seconds) by an ultrasonicator. The NPs were formed by lowering the temperature and solidifying the lipid matrix. Finally, the solidified NPs were centrifuged (20,000 rpm for 30 minutes at 4°C), and pelts were held in a -20°C freezer. The average size, polydispersity index, and entrapment efficiency were 158.1 ± 4.98 nm, 0.254 ± 0.06, and 70.09 ± 1.79, respectively.

Animals

In this study, 36 healthy adult male Wistar rats (weighing 200 ± 20 g) were obtained from the Laboratory Animal Maintenance, Reproduction, and Breeding Center of Hamedan University of Medical Sciences. The animals were housed at 23 ± 2°C with a 12-hour light/dark cycle and free access to a standard rodent diet and water. The protocol of conducting this study was confirmed by the Research Ethics Committee of Hamadan University of Medical Sciences (ethical code IR.UMSHA.REC.1400.634).

Experimental Design

The animals were randomly assigned to six experimental groups (6 rats per group), and 5 mg/kg/day of PQ was administered for seven consecutive days through oral gavage (22). After one hour, the same doses (100 mg/kg/day) of geraniol and nanogeraniol (NG) were administered through oral gavage in treatment groups (23). On the 8th day, all the animals were deeply anesthetized by ketamine/xylazine (8:1), and blood samples were drawn from the inferior vena cava. Moreover, half of the liver tissues of each group were kept at -80°C for biochemical studies, and the other half was fixed in 10% formalin for histological studies. In addition, blood samples were centrifuged (3,000 rpm for 10 minutes), and the serum was removed and kept at -20°C for further studies.

Liver Tissue Preparation

In general, 100 mg of liver tissue was homogenized in 1 mL of phosphate buffer (pH = 7.4, 50 mM), and after centrifugation at 3,000 rpm at 4°C for 10 minutes, the supernatant was removed and used to measure OS parameters (24).

Liver Function Assessment

The levels of serum liver enzymes, including AST, were determined according to the instructions of the kit (Pars Azmoun, Tehran kit, Iran).

Measurement of Lipid Peroxidation

The LPO of liver tissue was measured based on the reaction of malondialdehyde (MDA) with thiobarbituric acid. The MDA levels were estimated according to kit instructions (Kia Zist Company, Tehran, Iran). The maximum adsorption of the solution was read at 532 nm, and the concentration of MDA was reported as µmol/mg protein.

Determination of Total Antioxidant Capacity

This method is based on the capacity of samples to reduce Fe^{3+} to Fe^{2+} in the presence of TPTZ (10 mM). In this method, 20 µL of the sample was added to 200 µL of the ferric reducing antioxidant power reagent, vortexed, and incubated at 37°C for 4 minutes. Then, the optical absorption of the blue TPTZ-Fe^{2+} complex was read at a wavelength of 593 nm based on the calibration curve. The results were reported in µmol/mg protein (25).

Measurement of Total Thiol Molecule

The content of thiol groups in heart tissue was determined according to the method of Hu and Dillard (26). Briefly, 200 µL of the Tris-ethylenediaminetetraacetic acid (EDTA) buffer solution (0.25 M Tris, 2 mM EDTA, pH = 8.2) and 10 µL of the sample were mixed in a microplate well, and its initial absorbance was estimated at 412 nm. After adding 10 µL of the DTNB reagent and incubating the sample at 37°C for 15 minutes, the final absorbance of each sample and the DTNB blank was calculated at 412 nm. The results were reported in µmol/mg protein.

Total Protein Assay

At the end of each biochemical test, the amount of protein in the liver homogenate tissue was determined using the Bradford reagent at a wavelength of 595 nm against a standard curve prepared from bovine serum albumin.

Histological Studies

After fixing the liver tissue samples in 10% buffered formalin, paraffin blocks were prepared from the samples. Subsequently, sections with a thickness of 4 µm were prepared with a microtome and stained with the hematoxylin-eosin method. The slides were evaluated using an optical microscope (Olympus CX41) equipped with a digital camera (Olympus, DP25).

Statistical Analysis

The data analyzed by GraphPad Prism software (version 6.0) are reported as means ± standard deviations for all parameters. The statistical significance was estimated by one-way analysis of variance, followed by Tukey's test for multiple comparisons, and $P < 0.05$ was considered statistically significant.

Results

Liver Enzymes

PQ administration caused a significant increase in serum AST ($P < 0.001$) compared to the control group. On the other hand, treatment with 100 mg/kg of geraniol and NG noticeably decreased the serum level of AST ($P < 0.05$) in comparison with the control group (Figure 1).

Lipid Peroxidation

The cardiac MDA level considerably increased due to the administration of PQ for 7 consecutive days compared to the control group ($P < 0.001$). Although NG treatment could further decrease the MDA level, the administration of geraniol and NG at 100 mg/kg could significantly reduce the MDA level ($P < 0.001$) compared with the PQ group (Figure 2).

Antioxidant Defense System

Based on the results (Figure 3), the levels of TAC ($P < 0.05$) and TTG ($P < 0.01$) in liver tissue represented a considerable decrease following the oral administration of PQ at a dose of 5 mg for 7 consecutive days compared to the control group. However, only NG treatment at 100 mg/kg could significantly increase TAC ($P < 0.05$) and TTG ($P < 0.05$) compared to the PQ group.

Histological Changes

Examining the slides revealed that the control group (Figure 4A) had a normal tissue structure with hexagonal lobules. Further, the arrangement of hepatocytes was radial, and disc spaces were observed in the space between the liver cells. Furthermore, hepatocytes in this group had a large spherical nucleus with bright color and peripheral chromatin.

Following the exposure of rats to PQ (Figure 4B), the structure of the lobules was disordered, and the changes were in the form of infiltration of lymph cells around the central vein, irregularity in the arrangement of hepatocytes, and alterations in the size of hepatocytes. In addition, hepatocytes with dark and pyknotic nuclei and a very compact nuclear structure were observed in this group, indicating an increase in chromatin density and necrotic changes, respectively. The groups treated with NG (Figure 4C) and geraniol (Figure 4D) showed normal histological profiles. However, some of the hepatocytes in each lobule had dark and pyknotic nuclei, and the number of necrotic hepatocytes was slightly higher in the

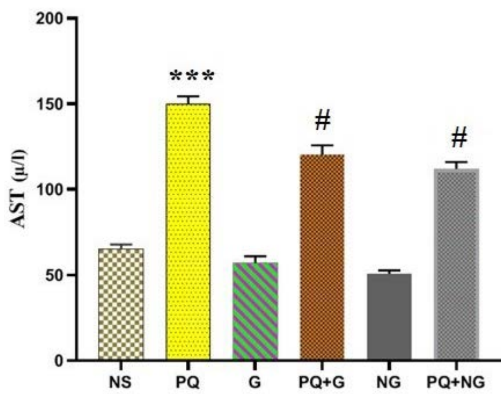

Figure 1. Effects of Geraniol and NG on Changes in AST Levels in PQ-Induced Hepatotoxicity
Note. The data were expressed as the mean ± standard error (n = 5). $^{***}P < 0.001$ compared to the control group, $^{\#}P < 0.05$ compared to the PQ group. NS: Normal saline. The control group received normal saline; PQ: Paraquat; G: Geraniol; NG: Nanogeraniol; AST: Aspartate aminotransferase.

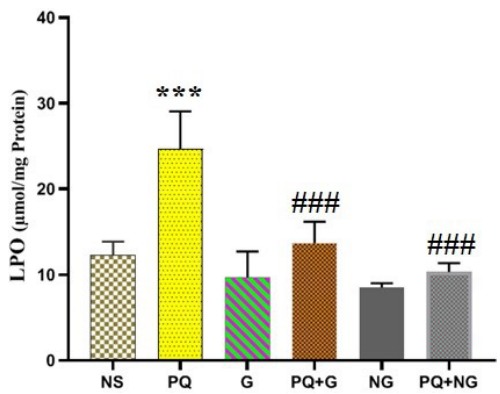

Figure 2. Effects of Geraniol and NG on Changes in MDA Levels in PQ-Induced Hepatotoxicity
Note. The data were expressed as the mean ± standard error (n = 5). $^{***}P < 0.001$ compared to the control group and $^{\#\#\#}P < 0.001$ compared to the PQ group. NS: Normal saline. The control group received normal saline, PQ: Paraquat; G: Geraniol; NG: Nanogeraniol; MDA: Malondialdehyde.

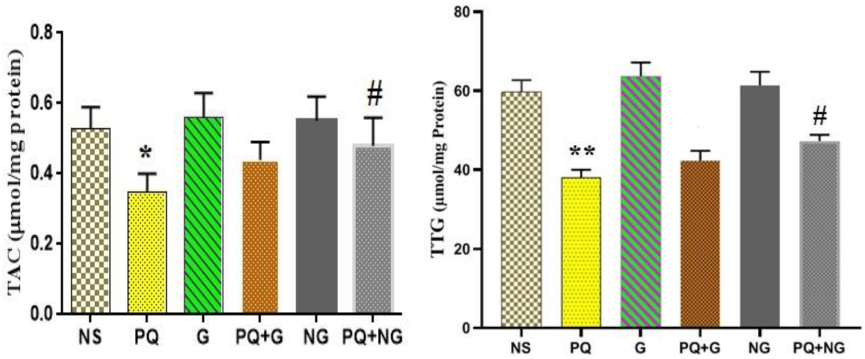

Figure 3. Effects of Geraniol and NG on Changes in TAC and TTG Levels in PQ-Induced Hepatotoxicity
Note. The data were expressed as the means ± standard error (n = 5). $^{**}P<0.01$ compared to the control group, $^{\#}P<0.05$ compared to the PQ group. NS: Normal saline. The control group received normal saline, PQ: Paraquat; G: Geraniol; NG: Nanogeraniol; TAC: Total antioxidant capacity; TTG: Total thiol molecule.

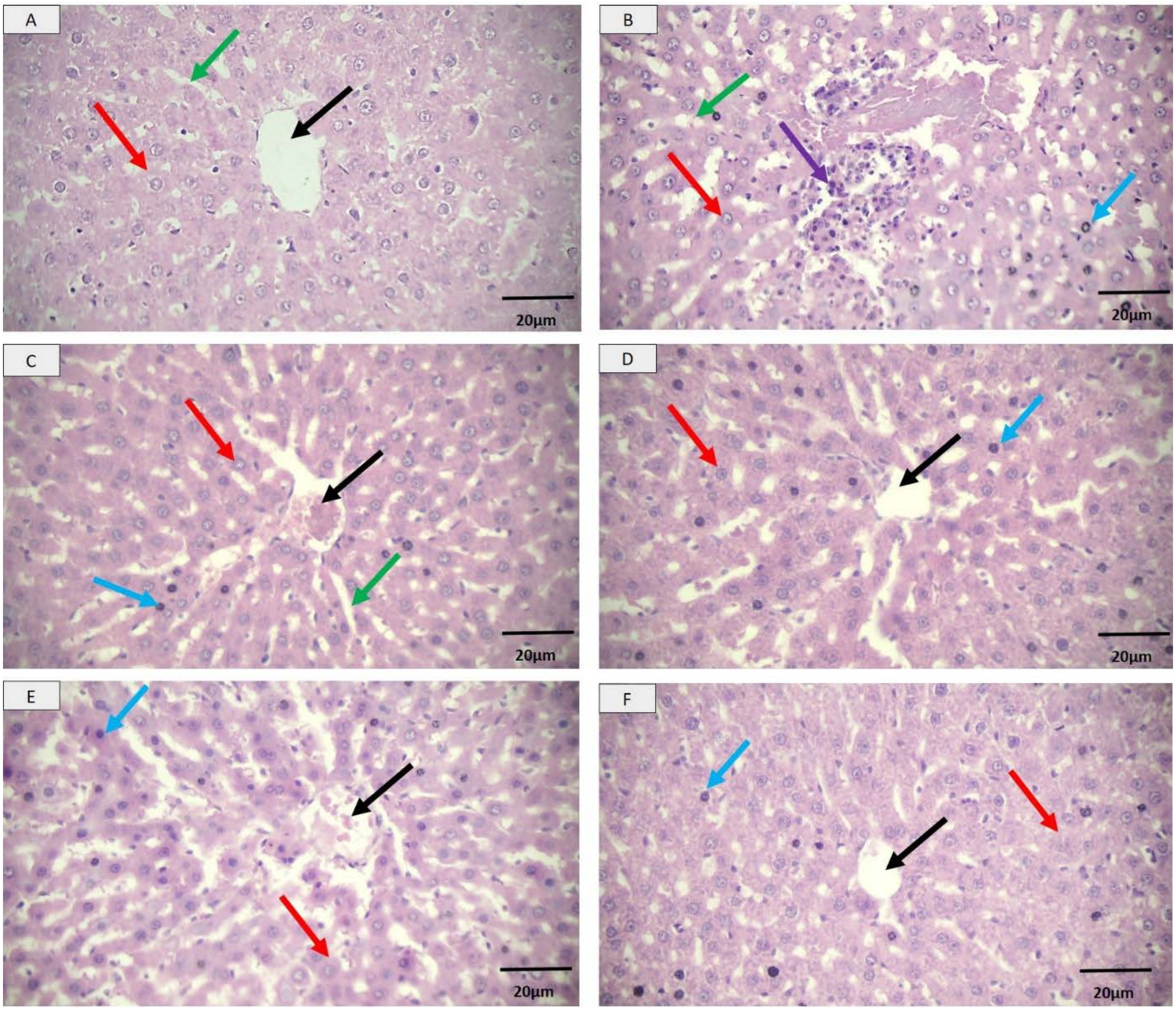

Figure 4. Hematoxylin-Eosin Staining—Histological Changes of the Liver From the Healthy Control Group **(A)**, PQ-Poisoned Group **(B)**, NG-Treated Group **(C)**, Geraniol-Treated Group **(D)**, PQ + Geraniol Group **(E)**, and PQ + NG Group **(F)**
Note. PQ: Paraquat; NG: Nanogeraniol. The images were selected as samples from the central vein region with 40x magnification. Black arrow: Central vein; Red arrow: Hepatocyte; Green arrow: Sinusoidal space or disc; Purple arrow: Lymphatic cells; Blue arrow: Pyknotic cells.

group receiving NG. Moreover, the PQ-poisoned groups treated with geraniol (Figure 4E) and NG (Figure 4F) both demonstrated an improvement in the histopathology of the liver, and NG NPs were more effective than geraniol. Thus, the NP form could reduce cellular disorganization and infiltration, thereby creating a tissue appearance similar to the normal state.

Discussion

In this study, the effects of hepatotoxicity in rats following 7 consecutive days of oral administration of PQ at a dose of 5 mg/kg were associated with marked changes in the parameters evaluated in the liver tissue enzyme function and redox system; these findings were well correlated with histological changes. Additionally, the experimental model of PQ was previously validated as a model of PQ-induced liver damage (27). Treatment with geraniol and NG reduced PQ-induced hepatotoxicity, reflecting the recovery following vitamin D administration, including the adjustment of the levels of liver enzyme function and biochemical markers in the OS, as well as the recovery of the induced histopathological damage via PQ. To the best of our knowledge, this is the first time that geraniol-loaded NLCs have been used to evaluate their hepatoprotective effectiveness.

The results of liver biomarkers confirmed that PQ administration increased the serum level of AST in animals, which is in line with the findings of Eftekhari et al (28). The ALT and AST enzymes in the cytoplasm and mitochondria of liver cells in the membrane of liver cells were several times higher than those of the extracellular fluid. When liver cells (e.g., the membrane structure of hepatocytes) are damaged, liver aminotransferase enzymes are released from the cell, and their serum concentration demonstrates an increase (29). Thus, it seems that PQ-treated rats experienced liver injury and the release of liver enzyme AST into the blood. On the other hand, geraniol and NG treatment markedly returned the AST level toward normal situations, and geraniol-loaded NLCs were more efficient than geraniol. Studies have shown that DDS is strongly believed to be one of the most interesting methods to enhance the bioavailability and therapeutic efficacy of drugs or bioactive compounds (30). In a rat model of liver ischemia-reperfusion injury, El-Emam et al reported that the liver enzyme function of ALT and AST significantly decreased in rats treated with geraniol (31), which conforms to our findings.

LPO is a vulnerable process against ROS. Due to high amounts of unsaturated fatty acids, liver tissue can also be prone to destruction and inflammation. The formation of reactive aldehydes (e.g., MDA and its increase) indicates that the cell membrane is attacked by ROS. Thus, cell membrane integrity, fluidity, ion transport, and enzyme activity are altered due to LPO and oxidative damage (32). In the present study, the oral administration of PQ for 7 days induced OS in the liver tissue by increasing the level of MDA as an index of LPO, which corroborates the findings of Rafiee et al (27). An imbalance of oxidant and antioxidant levels leads to OS. In normal conditions, the free radicals produced by the antioxidant system are converted into water and oxygen in the mitochondria (33).

Moreover, the content of thiol groups (TTM) and antioxidant capacity (TAC) in the liver tissue was significantly reduced in the group treated with PQ. TTM play an essential role in detoxifying substances and reducing OS in the body, and their inactivation can decrease the antioxidant system (34). After PQ enters the cell, it is converted to the PQ radical in the redox reaction. Subsequently, superoxide anions (O_2^-) will be generated because of the reaction between the PQ radical formed and molecular oxygen (35). Accordingly, concomitant cellular OS was manifested by increased ROS overproduction and reduced TAC and TTM levels (36).

Our previous study revealed that nanocurcumin and curcumin can modulate PQ-induced hepatotoxicity in rats. Additionally, the findings of this study showed that, compared to curcumin, nanocurcumin had greater hepatoprotective effects on liver damage after PQ exposure, most likely through the modulation of OS and gene expression of the nuclear factor erythroid 2-related factor 2 pathway (22).

On the other hand, the administration of geraniol and, more effectively, NG-loaded NLCs decreased the level of MDA in the liver tissue, which is in accordance with the observations of Li et al (37). In a rat model of aluminum chloride-induced hepatotoxicity, Hosseini et al found that geraniol (100 mg/kg, p.o.) could decrease the hepatic MDA level (23). Due to the antioxidant characteristics of geraniol, it seems that geraniol increases the activity of antioxidant enzymes by improving the content of TAC and TTM in liver tissue. Hence, geraniol acts as a radical scavenger, and the deleterious effects of PQ can be inhibited, leading to a reduction in the hepatic MDA level (14, 38). It should be noted that the encapsulation of geraniol into NLCs led to an increase in the antioxidant activity of geraniol, which may be because of bypassing the first-pass effect, which is one of the most important properties of NLC formulation (39). Therefore, NG-loaded NLCs were therapeutically more efficient than geraniol.

Histological studies confirmed the remarkable induction of liver damage after PQ administration in such a way that liver tissue damage included irregularity in the arrangement of hepatocytes and lobules and necrotic changes, which matches the results of Zhang et al (40). As mentioned above, PQ led to excessive production of ROS and stress damage, causing hepatic injuries (41). However, treatment with geraniol and NG could improve the histological structure of liver tissue, and the effectiveness of NG NPs was more than that of geraniol. Likewise, El Azab et al concluded that geraniol free radical scavenging and antioxidant activity reduced histological damage induced in carbon tetrachloride-treated rats (38). However, geraniol NPs could decrease cellular disorganization and infiltration, creating a hepatic tissue appearance identical to the normal state.

Conclusion

Overall, our findings confirmed the modulatory effects of geraniol and NG against PQ-induced hepatotoxicity. Considering the role of OS in hepatotoxicity caused by PQ, it seems that the NLC formulation of geraniol increased its antioxidant activity, leading to the inhibition of oxidative and hepatic damage. Therefore, geraniol-loaded NLCs may be a valuable candidate for inhibiting PQ-induced hepatic injuries. Nonetheless, further research is needed to support our findings.

Acknowledgements

This study was supported by the Vice-Chancellor for Research and Technology at Hamadan University of Medical Sciences (Grant No. 140008186853).

Competing Interests

The authors declared they have no conflict of interests.

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