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ORIGINAL ARTICLE

Zataria multiflora and its constituent, carvacrol, counteract sepsis-induced aortic and cardiac toxicity in rat: Involvement of nitric oxide and oxidative stress

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Abstract

Background: *Zataria multiflora* and carvacrol showed various pharmacological properties including anti-inflammatory and anti-oxidant effects. However, up to now no studies have explored its potential benefits in ameliorating sepsis-induced aortic and cardiac injury. Thus, this study aimed to investigate the effects of *Z. multiflora* and carvacrol on nitric oxide (NO) and oxidative stress indicators in lipopolysaccharide (LPS)-induced aortic and cardiac injury.

Methods: Adult male Wistar rats were assigned to: Control, lipopolysaccharide (LPS) (1 mg/kg, intraperitoneal (i.p.)), and *Z. multiflora* hydro-ethanolic extract (ZME, 50–200 mg/kg, oral)- and carvacrol (25–100 mg/kg, oral)-treated groups. LPS was injected daily for 14 days. Treatment with ZME and carvacrol started 3 days before LPS administration and treatment continued during LPS administration. At the end of the study, the levels of malondialdehyde (MDA), NO, thiols, and antioxidant enzymes were evaluated.

Results: Our findings showed a significant reduction in the levels of superoxide dismutase (SOD), catalase (CAT), and thiols in the LPS group, which were restored by ZME and carvacrol. Furthermore, ZME and carvacrol decreased MDA and NO in cardiac and aortic tissues of LPS-injected rats.

Conclusions: The results suggest protective effects of ZME and carvacrol on LPS-induced cardiovascular injury via improved redox hemostasis and attenuated NO production. However, additional studies are needed to elucidate the effects of ZME and its constituents on inflammatory responses mediated by LPS.

KEYWORDS
carvacrol, lipopolysaccharide, nitric oxide, oxidative injury, sepsis, *Z. multiflora*

1 | INTRODUCTION

Sepsis is characterized by inflammatory responses to infection and can be lead to death in critically ill individuals.[1] Activation of innate immunity and inflammatory responses by lipopolysaccharide (LPS), the main pathogenic factor of gram-negative bacteria, is responsible for the organ damage.[2] The stimulation of Toll-like receptor-4 by LPS results in activation of nuclear factor kappa ß (NF-kß). NF-κB regulates the expression of multiple inflammatory mediators.[2–4] LPS can directly trigger some acute inflammatory responses in endothelial cells including release of vascular cell adhesion molecule-1, E-selectin, intercellular adhesion molecule-1, pro-inflammatory cytokines (such as tumor necrosis factor-α (TNF-α), interleukin (IL)-6), and monocyte chemoattractant protein-1. During sepsis, systemic inflammation is regarded as a necessary host-defense response to pathogens. However, uncontrolled hyper-inflammatory responses through the release of various mediators result in refractory hypotension, cardiovascular hypo-reactivity, intravascular coagulation, and multiple organ failure.[3,4] The heart is one of the most adversly affected organs in the patients with sepsis. Sepsis-induced cardiovascular dysfunction can increase the incidence of mortality fourfold, compared with septic patients without cardiovascular disturbance.[3,5,6] Overproduction of reactive species such as nitric oxide (NO) is considered one of the predominant components of inflammatory events. A large number of studies have suggested that dysregulation of NO is involved in the pathophysiology of septic shock and organ damage.[5,7,8] In this regard, Tsai et al. showed that endotoxemia-provoked inflammatory responses and NO production were accompanied by cardiovascular disturbance.[8]

In vivo experiments have demonstrated that redox homeostasis is disturbed in LPS-injured hearts, as indicated by lowered antioxidant capacity and excess intracellular ROS.[3,9] Oxidative stress is a consequence of the imbalance between the production of ROS and antioxidant capacity. Enzymatic and non-enzymatic anti-oxidants quench ROS and maintain redox homeostasis. In response to oxidative damage, the cells up-regulate antioxidant enzymes like superoxide dismutase (SOD) and catalase (CAT). Mitochondria are considered to be the main site of intracellular ROS production, and are highly susceptible to oxidative stress. Excessive formation of ROS can alter mitochondrial membrane permeability, leading to apoptotic cell death.[5,6,10] Considering the role of oxidative injury in sepsis-induced toxicity, utilizing compounds with antioxidant potential could be a promising approach to prevention of LPS-induced organ injury.[3,11]

Medicinal herbs have long been considered as a helpful strategy for the management of various ailments from ancient times to the present.[12,13]

Zataria multiflora, belonging to Lamiaceae family, is native to south-western Asia.[14,15] *Z. multiflora* has been used in traditional medicine as an antiseptic, carminative, stimulant, diaphoretic, diuretic, anesthetic, anti-spasmodic, and analgesic agent.[15] In addition, this medicinal plant has been found to have antimicrobial, anti-oxidative, anti-inflammatory, spasmolytic, and anti-nociceptive properties. Various bioactive compounds such as phenolic (thymol, carvacrol, and linalool) and non-phenolic (p-cymene, γ-Terpinene, and α-Pinene) have been found in *Z. multiflora*.[14,15] Carvacrol is an oxygenated monoterpene found in high concentrations in *Z. multiflora*.[14] A number of studies have focused on the anti-inflammatory potential of *Z. multiflora* and carvacrol.[15,16] In this context, Boskabady et al. showed that treatment with a hydro-ethanol extract of *Z. multiflora* (ZME) decreased lung inflammation in an animal model of chronic obstructive pulmonary disease (COPD).[17] In another study, administration of carvacrol (40–160 μg/mL, oral) to sensitized animals reduced release of some inflammatory mediators including IL-4, endothelin, prostaglandin E2, and IL-1β.[18] Carvacrol was also found to alleviate the mechanical hypernociception and inflammation induced by carrageenan and TNF-α.[19] In human macrophage-like U937 cells, carvacrol suppressed LPS-induced cyclooxygenase-2, suggesting its anti-inflammatory potential.[20] Several other lines of evidence suggest the antioxidant activities of *Z. multiflora* and carvacrol.[14,15,21] However, the specific protective mechanisms of ZME and carvacrol have not yet been assessed in animal models of LPS-induced sepsis. This study aimed to evaluate the probable effects of ZME and carvacrol on the markers of oxidative stress and NO in cardiac and aortic injury induced by LPS in rat.

2 | METHODS

2.1 | Plant material and extraction

The aerial parts of *Z. multiflora* were obtained from Khorasan Razavi province, Iran. This plant was identified by an expert botanist in the herbarium of Ferdowsi University of Mashhad, Mashhad, Iran. *Z. multiflora* aerial parts were air dried in the shade and ground to a fine powder. The extract was made using the maceration method.[22] For this purpose, the dried powder (100 g) was soaked in a hydro-ethanolic solution (70%, v/v, 1000 mL) for 72 h. Following that, the extract was filtered using No. 1 Whatman filter paper and centrifuged for 5 min at 10000×*g*. The supernatant was concentrated (at 37°C) and kept at −20°C. The yield of extraction was 33.2% w/w of the dried powder.

2.2 | Animals and treatment protocols

Eighty adult male Wistar rats (weighing 200–220 g) were obtained from the animal laboratory of Mashhad University of Medical Sciences (Mashhad, Iran). After acclimatization for 1 week, the animals were randomly assigned to the following eight groups ($n = 10$ per group).

Group 1 (control) in which the healthy animals received vehicle (saline, 1 mL/kg, plus Tween, 0.2%, oral) instead of ZME and carvacrol, and saline (1 mL/kg) was injected (intraperitoneal (i.p.)) instead of LPS.

Group 2 (LPS) in which the animals were treated with vehicle (saline, 1 mL/kg, plus Tween, 0.2%, oral) and LPS (1 mg/kg, i.p.).[11]

Groups 3–8 (LPS-ZME 50, LPS-ZME 100, LPS-ZME 200, LPS-car 25, LPS-car 50, and LPS-car 100) in which the animals received ZME (at doses of 50, 100, and 200 mg/kg, oral) and carvacrol (at doses of 25, 50, and 100 mg/kg, oral), respectively. After 30 min, LPS (1 mg/kg, i.p.) was injected.

Doses of ZME[23,24] and carvacrol[15,24,25] were selected based on previous investigations.

The required concentrations of carvacrol and ZME for these experiments were dissolved in saline diluted with Tween 80 (0.2% v/v). It should be noted that the doses of carvacrol and ZME were adjusted daily based on the body weights of the animals.

In the current study, the treatment groups (groups 3–8) were orally given ZME and carvacrol, whereas the control group (group 1) received the vehicle for 17 days. Group 2 (LPS) was given LPS for 14 days. Accordingly, treatment with ZME and carvacrol started 3 days before LPS administration and the treatments continued during LPS administration. All treatments were administered once per day (at 10:00 am).

During the experiments, the animals were kept under standard laboratory conditions (12-h dark/12-h light period, 23 ± 1°C temperature, and 50%–60% humidity), with free access to food and water ad libitum. All experimental procedures followed the relevant guidelines and regulations of the National Institute of Health as outlined in the *Guide for the Care and Use of Laboratory Animals* (NIH Publications No. 80-23, revised 1978) and were approved by the Ethics Committee of Mashhad University of Medical Sciences, Iran (IR.MUMS.fm.REC.1397.139). At the end of the treatment period, all rats were deeply anesthetized with ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.). Following that, the rats were decapitated and the cardiac and aortic tissues were transferred to a freezer (−80°C). In order to prepare a tissue homogenate (10% w/v), whole-aorta sections and equal proportions (200 mg) of heart tissues (left ventricle) were suspended in physiological saline solution (2 mL, 0.9%). The corresponding supernatants were separated for the biochemical experiments.

2.3 | Lipid peroxidation

In order to estimate the status of lipid peroxidation, the concentration of MDA was determined. For this purpose, 500 mL of tissue homogenate was added to a tube containing 2-thiobarbituric acid (0.375%), trichloroacetic acid (15%, TCA), and 2 mL of hydrochloric acid. Following that, this mixture was heated at 95°C. After boiling for 45 min, the mixture was allowed to cool to ambient temperature. Finally, the mixture was centrifuged (at 10000×*g*) for 10 min. The absorbance of each supernatant was recorded at 535 nm. The concentration of MDA was expressed as nmoles per tissue weight (g).[22]

2.4 | Non-enzymatic antioxidants

In order to estimate the non-enzymatic antioxidants, the concentration of the thiols (or sulfhydryl groups) in the cardiac and aortic sections was measured using 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) reagent. For this experiment, each sample (50 µL) was mixed with tris-ethylenediaminetetraacetic acid (EDTA) buffer (1 mL, pH 8.26) and the absorbance was recorded (*A*1). DTNB reagent (20 µL, 10 mmoL/L) was then dispensed into the tubes. The reaction of sulfhydryl groups with DTNB produces a yellow-colored complex. Finally, the second absorbance (*A*2) was read at 412 nm. The absorbance of the DTNB reagent was also recorded (*B*). In order to calculate the concentration of thiols, the following equation was used.[22]

$$\text{Total thiol concentration (mmol/L)} = (A2 - A1 - B) \times 1.07/(0.05 \times 13.6).$$

2.5 | Enzymatic antioxidant systems

SOD activity in the cardiac and aortic sections was measured as previously described.[22] For this purpose, a sample (60 µL) was dispensed into each well of a 96-well plate containing PBS (60 µL). Following that, pyrogallol solution (15 µL, 0.1 mg/mL) and MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium; 6 µL, 0.5 mg/mL) were added. The plate was then incubated for 5 min at room temperature. SOD enzyme scavenges free radicals generated following pyrogallol autoxidation. Finally, dimethyl sulfoxide (100 µL) was added to solubilize the formazan crystals. The absorbance at 570 nm was noted and the SOD activity was expressed as units per tissue weight (g).

In order to estimate the CAT activity, 50 µL of each sample was added to phosphate buffer (200 µL) and hydrogen peroxide (H_2O_2, 250 µL, 0.06 moL/L). One unit of CAT activity is defined as the amount of SOD concentration degrading H_2O_2 per minute. The reduction in absorbance was monitored using an UV–visible spectrophotometer at 240 nm per min.[22,26]

2.6 | Estimation of NO metabolites (nitrite/nitrate)

The total nitrite and nitrate concentration in tissue homogenates was measured to determine the NO concentration. For this purpose, each sample (100 µL) was mixed with Griess reagent. Following that, 50 µL of TCA (10%) was added to each tube. In order to separate the supernatants, the mixtures were centrifuged at 16000×*g* for 5 min. Finally, the absorbance at 520 nm was noted. The concentrations of NO in cardiac and aortic tissues were calculated after comparison with standard solutions of sodium nitrate.[27]

2.7 | Statistical analysis

Prism version 9 was used for data analysis. Data were presented as means ± SEM. $p < 0.05$ was considered as statistically significant. Differences between the experimental groups were assessed by one-way analysis of variance (ANOVA), followed by Tukey's post hoc comparison test.

3 | RESULTS

3.1 | ZME and carvacrol restore redox homeostasis in LPS-injured rats

3.1.1 | Lipid peroxidation

As shown in Figure 1 (panels A and B), the content of MDA was significantly increased in the cardiac (a) and aortic (b) tissues of the LPS-injected rats compared to the control group ($p<0.01$ and $p<0.001$ for heart and $p<0.001$ for aorta). However, treatment with ZME and carvacrol significantly decreased the content of MDA in both heart and aorta compared to LPS-injected rats (ZME: $p<0.01$ and $p<0.001$ for heart, $p<0.001$ for aorta. Carvacrol: $p<0.05$ and $p<0.01$ in heart, $p<0.001$ in aorta). Notably, MDA level was found to be greater in cardiac tissue of the animals treated with 50, 100, and 200 mg/kg of the extract, compared to the control group ($p<0.001$).

3.1.2 | Antioxidants

Determining the thiol content indicated the non-enzymatic capacity of ZME and carvacrol against the oxidative stress. The thiol concentrations in both the heart and aorta of LPS-injected rats were significantly lower than those of the control group (Figure 2; $p<0.05$ and $p<0.001$; panels A and B). As shown in Figures 3 and 4, the antioxidant enzyme (SOD and CAT) activities were significantly attenuated in the heart and aorta of the LPS group compared to those of the controls (SOD, $p<0.001$ for all. CAT, ZME: $p<0.01$ and $p<0.001$ for heart and aorta; Carvacrol: $p<0.001$ for heart). However, treatment of these animals with carvacrol (at 25, 50, and 100 mg/kg) and ZME (50, 100, and 200 mg/kg) significantly increased the levels of SOD, CAT, and thiols in the heart and aorta compared to those of the LPS group (Figures 2–4. SOD, ZME: $p<0.01$ and $p<0.001$ for heart and $p<0.001$ for aorta, carvacrol: $p<0.05$ and $p<0.01$ for heart and $p<0.01$ and $p<0.001$ for aorta. CAT, ZME: $p<0.05$ and $p<0.01$ for heart and $p<0.001$ for aorta, carvacrol: $p<0.05$ in heart. Thiols, carvacrol: $p<0.01$ and $p<0.001$ for heart and $p<0.05$ and $p<0.001$ for aorta). The results showed that thiol levels in the aortic tissues of LPS-ZME 50 ($p<0.05$), LPS-car 25, and LPS-car 50 groups ($p<0.001$) were still higher than those of the control group. Furthermore, the SOD level was found to be lower in the cardiac tissue of the animals treated with 25 mg/kg of carvacrol relative to the control group ($p<0.01$). Notably, in heart some significant differences in SOD activity and total thiol content were found between LPS-ZME 50 and LPS-ZME 200 groups ($p<0.05$), as well as between LPS-ZME 50 and control groups ($p<0.01$). Similarly, in aorta some significant differences in CAT activity were found between LPS-ZME 50 and LPS-ZME 200 groups ($p<0.01$), as well as between LPS-ZME50 and control groups ($p<0.001$).

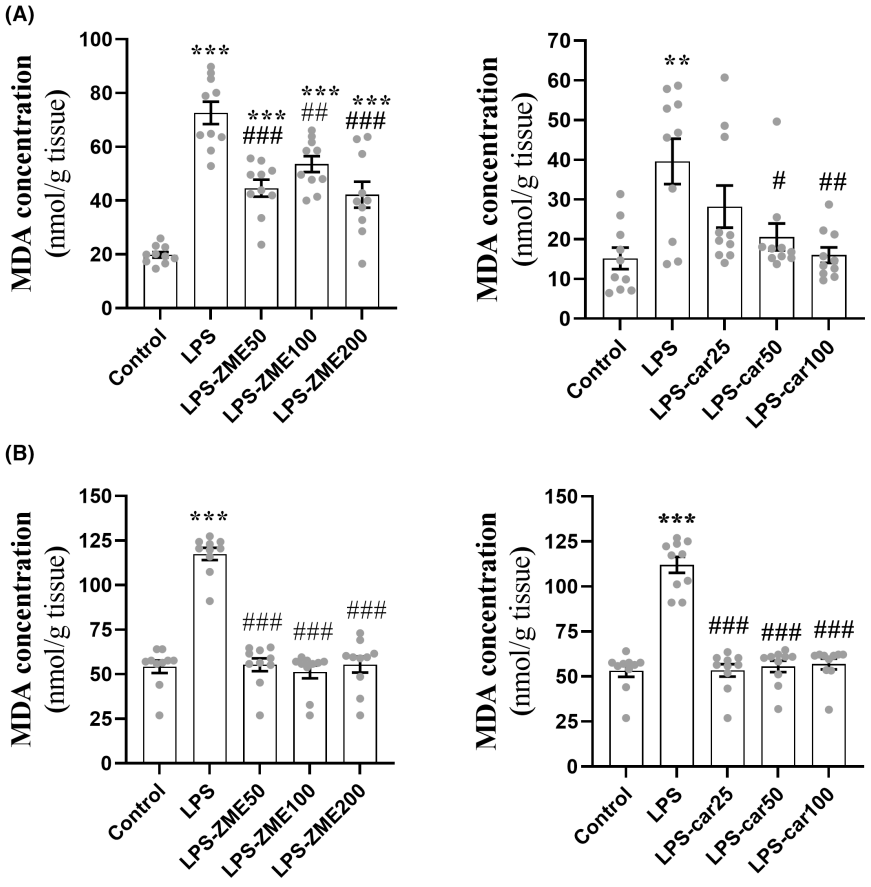
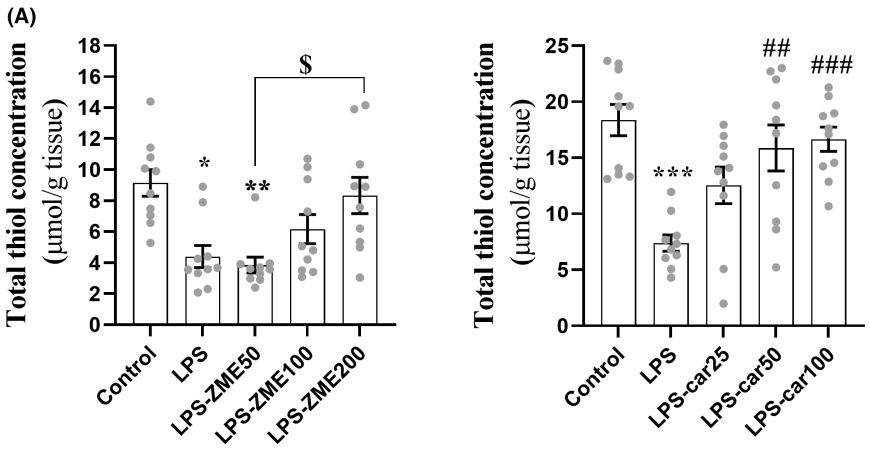


FIGURE 1 Effects of *Z. multiflora* extract (ZME) and carvacrol (car) on malondialdehyde (MDA) in heart (A) and aorta (B) of lipopolysaccharide (LPS)-injected rats. Data are expressed as mean ± SEM ($n=10$). $^{**}p<0.01$ and $^{***}p<0.001$ compared to control group. $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$ compared to LPS group.



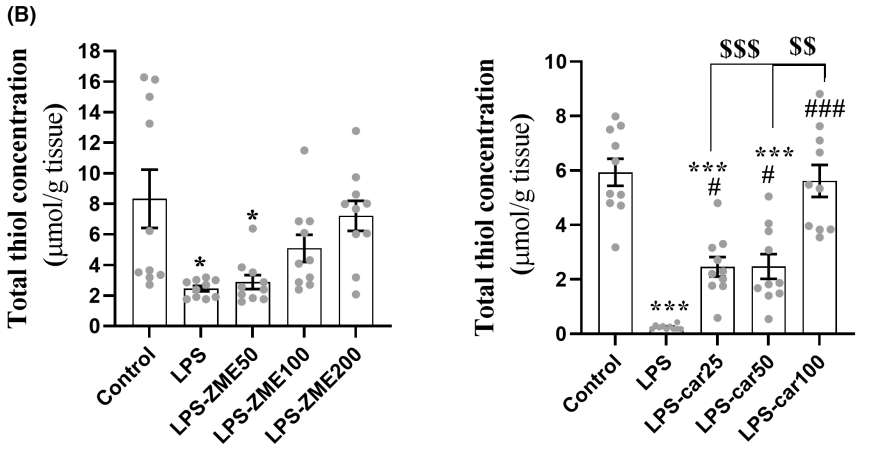
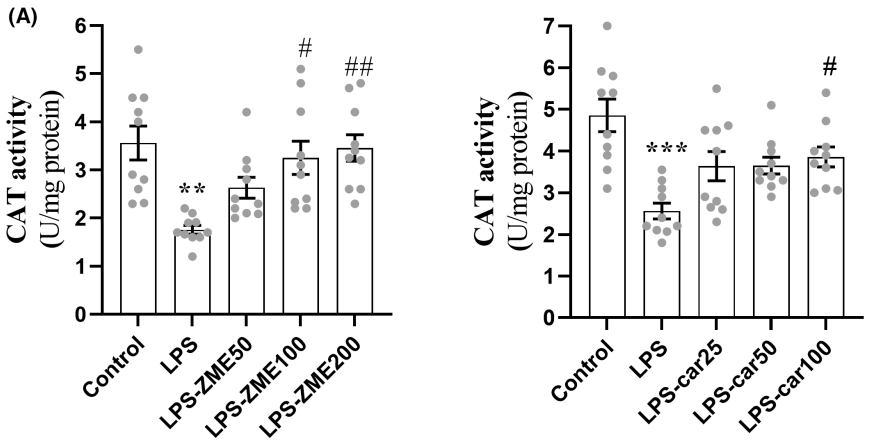


FIGURE 2 Effects of *Z. multiflora* extract (ZME) and carvacrol (car) on the thiol groups in heart (A) and aorta (B) of lipopolysaccharide (LPS)-injected rats. Data are expressed as mean ± SEM ($n = 10$). $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared to control group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to LPS group. $^{\$\$}p < 0.01$ and $^{\$\$\$}p < 0.001$ comparison between the treatment groups.



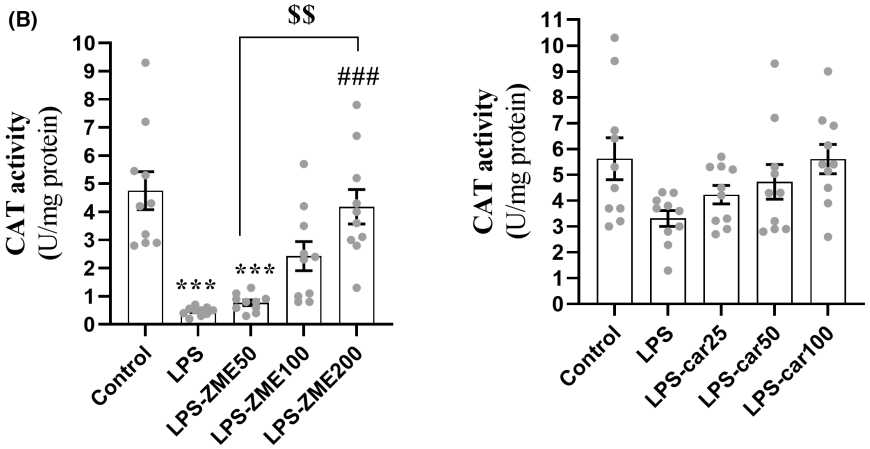


FIGURE 3 Effects of *Z. multiflora* extract (ZME) and carvacrol (car) on catalase (CAT) activity in heart (A) and aorta (B) of lipopolysaccharide (LPS)-injected rats. Data are expressed as mean ± SEM ($n = 10$). $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to control group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to LPS group. $^{\$\$}p < 0.01$ comparison between the treatment groups.

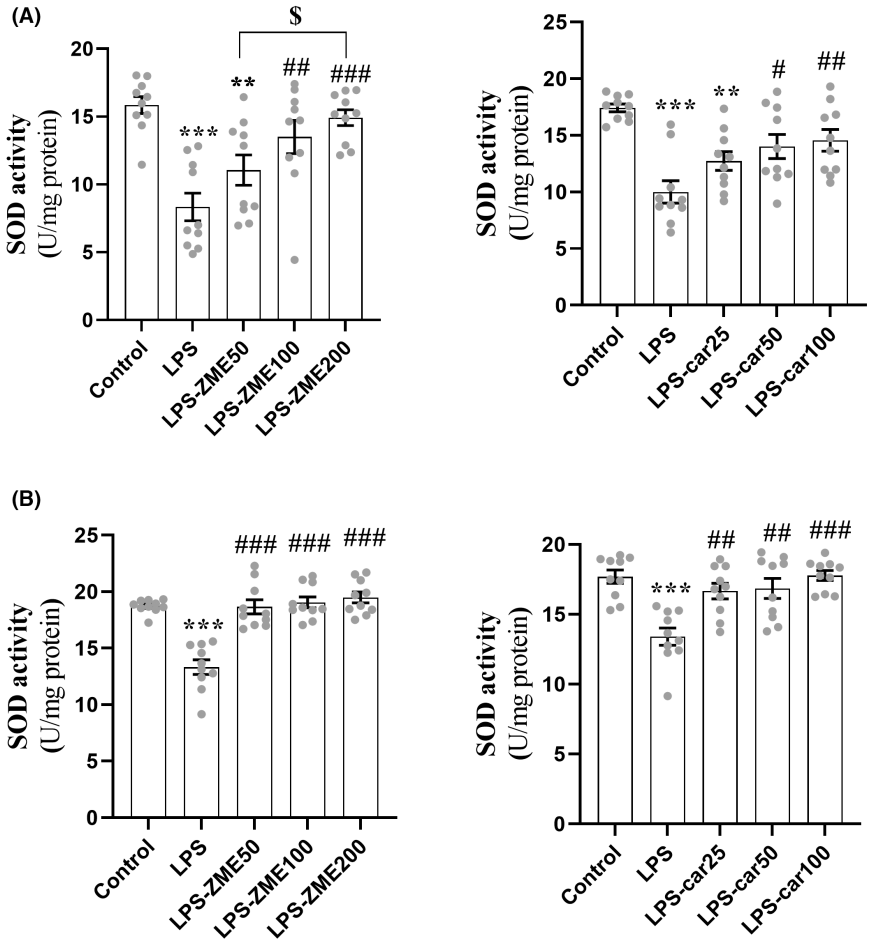


FIGURE 4 Effects of *Z. multiflora* extract (ZME) and carvacrol (car) on superoxide dismutase (SOD) activity in heart (A) and aorta (B) of lipopolysaccharide (LPS)-injected rats. Data are expressed as mean ± SEM ($n = 10$). $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to control group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to LPS group. $^{\$}p < 0.05$ comparison between the treatment groups.

3.1.3 | ZME and carvacrol decrease NO level in LPS-injured rats

The results showed a significant increase in the NO content in the cardiac and aortic tissues of the LPS-injected rats compared to the control group (Figure 5; $p < 0.001$ for all). However, ZME and carvacrol supplementation significantly decreased the NO content in the cardiac and aortic tissues compared to that of the LPS group (ZME: $p < 0.05$ and $p < 0.01$ for heart, $p < 0.05$ and $p < 0.001$ for aorta, carvacrol: $p < 0.05$ and $p < 0.01$ for heart and aorta). Notably, the NO level was found to be greater in aortic tissue of the animals treated with 50 mg/kg of the extract, compared to the control group ($p < 0.01$).

4 | DISCUSSION

The results of the current study suggested that ZME and carvacrol improve cardiac and aortic injury in the LPS-injected rats, as well as modifying NO production and oxidative stress markers including MDA, thiols, SOD, and CAT.

LPS-induced inflammation involves several cell types such as lymphocytes, macrophages, and granulocytes.[3] Activation of immune responses is associated with production of multiple pro-inflammatory cytokines and adhesion molecules which have detrimental effects on several organs.[2–4] LPS-induced systemic inflammation has also been shown to disturb endothelial vascular homeostasis with subsequent irreversible cardiac dysfunction.[3]

Over-production of reactive species including NO represents one of the predominant components of inflammatory events.[7,8] NO, produced by inducible NO synthase,[7] can exacerbate inflammatory responses as well as medicate nitrosative stress.[3,28–30] Many studies have indicated an increase in the level of NO after LPS injection.[8,31,32]

In agreement with previous studies,[8,31,32] the data from our study showed that systemic administration of LPS significantly increased the content of NO in both cardiac and aortic tissues.

Previous studies revealed that *Z. multiflora* and carvacrol induced immunomodulatory and anti-inflammatory effects in experimental models of inflammatory bowel syndrome, COPD, and asthma.[16,33,34] For instance, a recent study by Amin et al. showed that treatment with ZME (200 and 400 mg/kg) modified the levels of nitrite, IL-17, TNF-α, IL-10, and interferon-gamma in systemic inflammation induced by paraquat in rats.[34] Carvacrol and *Z. multiflora* essential oil were also shown to suppress arachidonic pathways and NO production in LPS-stimulated macrophages.[20,35]

The results of the current research showed that ZME and carvacrol reduced the level of NO, as an inflammatory mediator. However, further research should be performed to explore the impact of ZME

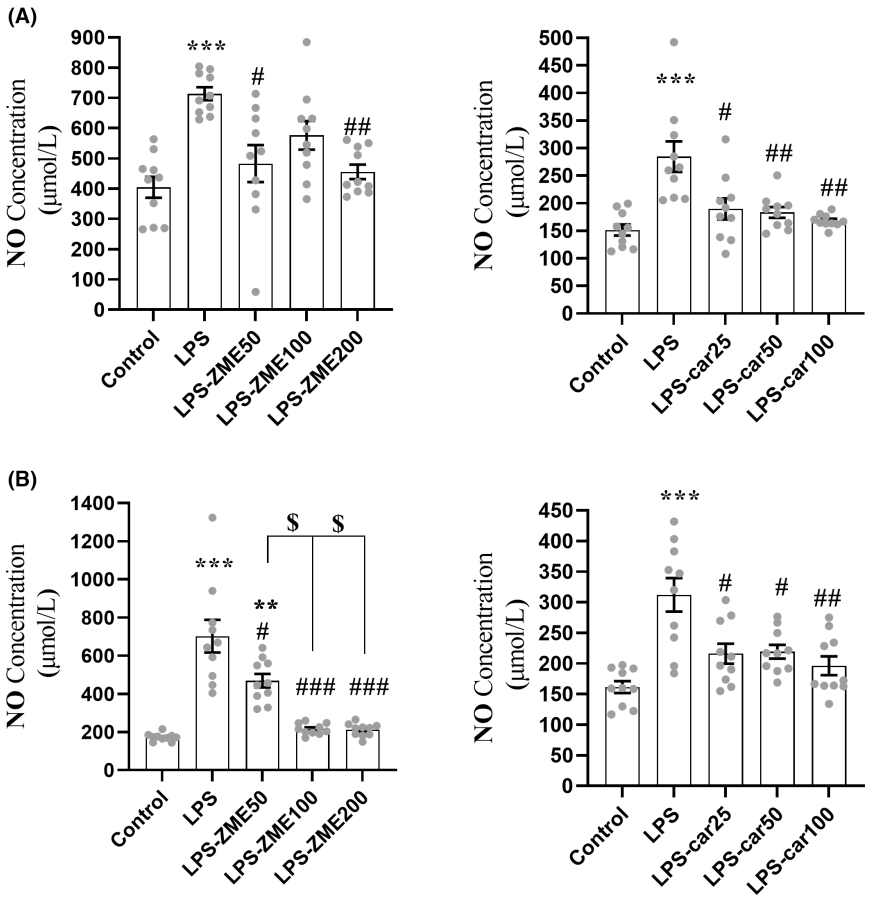


FIGURE 5 Effects of *Z. multiflora* extract (ZME) and carvacrol (car) on nitric oxide (NO) in heart (A) and aorta (B) of lipopolysaccharide (LPS)-injected rats. Data are expressed as mean ± SEM ($n = 10$). $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to control group. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$ compared to LPS group. $^{\$}p < 0.05$ comparison between the treatment groups.

and carvacrol on pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) in LPS-treated animals.

Growing evidence has revealed that inflammation and oxidative stress are closely linked. ROS promote the release of pro-inflammatory mediators. The process in turn produces more ROS.[5,6,9] The vicious cycle has a detrimental effect on tissues by mediating mitochondrial disturbance, aggravating cellular damage and eventually leading to chronic diseases.[5,9]

In the current study, oxidative stress markers including MDA, total thiol groups, CAT, and SOD were determined. CAT and SOD are considered the first line of cell defense against oxidative stress.[5,10]

According to our data, LPS injection was associated with a significant increase in the level of MDA while decreasing the levels of thiols, SOD, and CAT in both heart and aorta. These results were consistent with previous studies.[9,36] A previous study showed that LPS injection induced a noticeable increase in lipid peroxidation with subsequent depletion of antioxidants in the cardiovascular system.[36] Peroxidation of lipids is likely due to ROS interactions with membranous polyunsaturated fatty acids.[9,10]

As mentioned earlier, LPS-induced acute myocardial injury is considered a main characteristic of sepsis.[1,9,36] A growing body of literature suggested that oxidative/nitrosative stress disrupts the architecture of cardio-myocytes during sepsis.[3,6,9] The loss of structural integrity of the cardio-myocytes is reflected by abnormal serum levels of biochemical markers including aspartate aminotransferase (AST), cardiac muscle creatinine kinase (CK-MB), troponin T (cTn-T), lactate dehydrogenase (LDH), and B-type natriuretic peptide (BNP).[9,37] Previous studies revealed a significant disturbance in these biochemical factors, associated with a reduction in antioxidant capacity in LPS-injured hearts.[9,37]

A limitation of this study was that the markers of cardiac toxicity were not assessed. Additional research should be conducted to assess the impact of ZME and carvacrol on the serum levels of AST, CK-MB, cTn-T, LDH, and BNP in the LPS-injured rats.

Our results showed that administration of ZME and carvacrol to the LPS-treated rats can increase the levels of thiols, CAT, and SOD, as well as decrease the content of MDA. In line with our results, previous investigations have shown that *Z. multiflora* and carvacrol counteract oxidative stress.[17,38–40] In this context, Khajavi et al. suggested that *Z. multiflora* extract and carvacrol would have protective effects against adriamycin-induced oxidative injury in heart tissue.[39]

The antioxidant and free radical scavenging activities of *Z. multiflora* and carvacrol have been previously shown.[15,21] Numerous investigations have revealed that compounds with antioxidant properties can attenuate LPS-induced organ damage.[1,9,36] Hence, the positive effects of ZME and carvacrol in the present study may partly be due to its antioxidant effects.

Considering the role of oxidative stress in the development of inflammation, the relief of oxidative stress can be presumed to have suppressed NO production in this study.

Furthermore, we speculate that attenuation of LPS-induced cardiovascular injury by ZME may be attributed to the presence of bioactive compounds like carvacrol.

Preclinical and clinical studies indicate that there are no major treatment-related adverse effects following administration of the hydro-ethnolic extracts of this plant.[23,24,41,42] In this context, oral administration of the extract induced no noticeable change in body weight, hematological parameters, serum biochemical factors, and histopathology, even at a dose of 500 mg/kg.[23,41] Accordingly, short-term use of the extract does not seem to produce any significant toxicity. However, a limiting factor of this research was the lack of toxicity and safety investigations of ZME, and these should be done in future studies.

5 | CONCLUSION

The results of the present study showed that ZME and carvacrol protected rat cardiac and aortic tissues from LPS-induced injury. Possible mechanisms seem to be the ameliorative effect of ZME and carvacrol on NO and redox hemostasis. These findings provide evidence that ZME has potential protective effects against sepsis-induced cardiovascular damage.

AUTHOR CONTRIBUTIONS

Conceptualization, Mahmoud Hosseini and Arezoo Rajabian Supervised the project and designed the experiments. Zohreh Arab, Farimah Beheshti, Akbar Anaeigoudari, Farzaneh Shakeri, and Arezoo Rajabian; performed the experiments. Arezoo Rajabian wrote the manuscript and perfumed data analysis.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data presented in this study are available in the graph and tables provided in the manuscript.

ETHICS STATEMENT

All experimental procedures pursued the relevant guidelines and regulations of the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, revised 1978) and were approved by the Ethics Committee of Mashhad University of Medical Sciences, Iran (IR.MUMS.fm.REC.1397.139).

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