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Research Paper

Hepatoprotective Effects of Carvacrol on Arsenic Trioxide–Induced Liver Toxicity in Rats

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ABSTRACT

Background: Arsenic trioxide (As_2O_3, ATO) is commonly used to treat acute promyelocytic leukemia (APL); however, a major challenge in its use is hepatotoxicity, which has gained increasing attention in recent years. Many patients undergoing ATO therapy experience varying degrees of liver damage. Since oxidative stress plays a key role in ATO-induced liver injury, this study aimed to explore the potential protective effects of carvacrol on ATO-induced liver damage in rats.

Methods: In this study, rats were treated with ATO (5 mg/kg) either alone or in combination with carvacrol at doses of 50, 100, or 200 mg/kg. At the end of the treatment, several liver injury biomarkers, including ALT, AST, LDH, and bilirubin, were measured. Additionally, oxidative stress indicators in liver tissue, such as lipid peroxidation, antioxidant capacity, ROS levels, and glutathione (GSH) content, were assessed.

Results: Arsenic trioxide treatment led to significant liver damage, marked by increased levels of liver injury biomarkers (ALT, AST, and LDH), along with disruptions in oxidative stress markers. These changes included increased lipid peroxidation, elevated ROS levels, and decreased liver GSH content and antioxidant capacity. Importantly, carvacrol treatment at all tested doses effectively reduced these harmful effects, highlighting its potential as a hepatoprotective agent.

Conclusion: Carvacrol exhibits strong antioxidant activity and has the capacity to effectively counteract ATO–induced oxidative liver injury.

Keywords: Arsenic Trioxide, Carvacrol, Chemical and Drug Induced Liver Injury, Rats

Introduction

Cancer refers to a group of diseases characterized by abnormal cell growth and the ability to metastasize to other parts of the body. Cancer risk is primarily influenced by lifestyle factors, such as tobacco use, poor diet, physical inactivity, and alcohol consumption, along with genetic predispositions and certain infections [1]. Cancer presents with diverse symptoms, and its diagnosis generally relies on imaging modalities and histopathological evaluation through biopsy. Preventive strategies include quitting smoking, adhering to a healthy diet, engaging in regular physical activity, and receiving appropriate vaccinations. Cancer treatment options include surgery, radiation therapy, chemotherapy, and targeted therapeutic approaches [2]. Cancer encompasses various types, with lung, prostate, and colorectal cancers being most common in men, and breast, colorectal, and lung cancers being most prevalent in women. Global estimates indicate that the economic burden of cancer between 2020 and 2050 may reach US$25.2 trillion [3]. According to 2019 statistics, approximately 23.6 million new cancer cases were diagnosed annually, with around 10 million deaths attributed to the disease. This corresponds to a 26% increase in cancer incidence and a 21% rise in mortality over the past decade [4].

Cancer is not a single disease but a group of many different diseases. These are usually classified based on the type of cell in which the cancer began. The main categories are carcinomas (from epithelial cells), sarcomas (from connective tissues, such as bone or muscle), leukemias (from blood-forming cells in the bone

marrow), and lymphomas or myelomas (from immune or lymphoid cells). This classification helps guide the diagnosis and treatment of cancer [5]. Leukemia is a cancer originating in the bone marrow, characterized by the excessive proliferation of abnormal white blood cells. Common symptoms of leukemia include fatigue, abnormal bleeding, fever, and increased susceptibility to infections. Leukemia is classified into two main types: lymphoid and myeloid, with treatment options including chemotherapy, radiation therapy, and bone marrow transplantation. Early diagnosis and recent therapeutic advances have markedly improved the life expectancy of patients [6].

Arsenic trioxide (ATO) is a chemical agent employed in the treatment of acute promyelocytic leukemia (APL). It promotes patient recovery by inducing apoptosis in cancer

Blood and Tissue Collection

The rats were anesthetized intraperitoneally with thiopental (60 mg/kg), and 1–1.5 mL of blood was carefully collected via cardiac puncture. Samples were transferred to test tubes to prevent hemolysis and centrifuged at 3,000 rpm for 20 minutes at room temperature. The serum was separated and stored at −80°C until analysis of the desired parameters. Additionally, liver tissues were harvested for assessment of oxidant and antioxidant activities.

Determination of Biochemical Parameters and Oxidative Stress

Liver injury markers, including serum lactate dehydrogenase (LDH), aspartate aminotransferase (AST), and alanine aminotransferase (ALT), were measured using commercial kits (Pars Test). Oxidative stress in liver tissue was assessed by evaluating antioxidant capacity, ROS production, lipid peroxidation via the thiobarbituric acid reactive substances (TBARS) assay, and GSH levels using the 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) assay [14-16].

Determination of Oxidative Stress Markers

Tissue lipid peroxidation was measured using the Nalondi™ Malondialdehyde (MDA) Lipid Peroxidation Assay Kit. The assay is based on the reaction of thiobarbituric acid (TBA) with peroxidized lipids, producing MDA, which subsequently reacts with TBA to form a chromogenic product. The resulting product was quantified spectrophotometrically against a standard curve [17, 18].

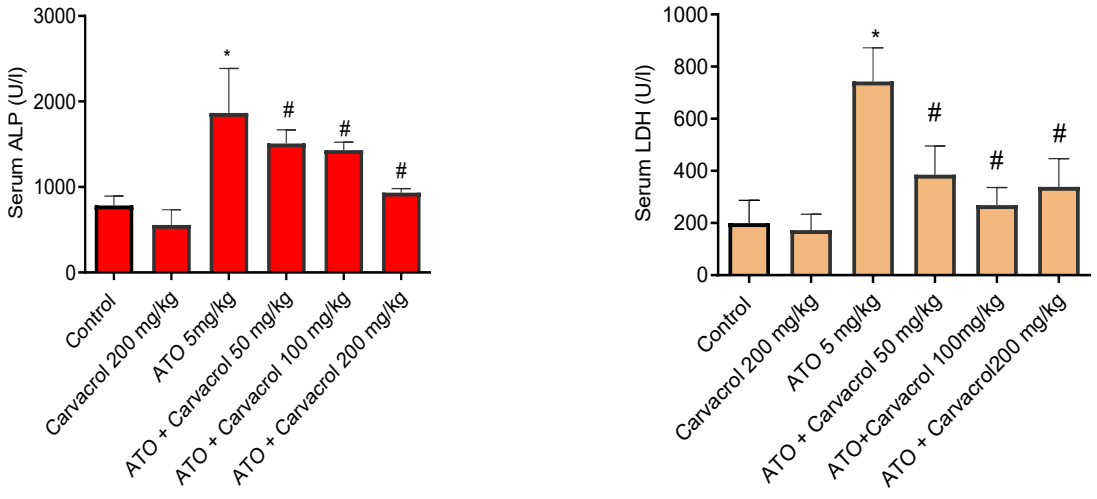
Statistical Analysis

Data were analyzed using the SPSS (version 24) software. Descriptive statistics were calculated to determine measures of central tendency and dispersion. One-way analysis of variance (ANOVA) was applied for normally distributed data, followed by Tukey's post hoc test for pairwise comparisons when significant differences were observed. For non-normally distributed data, the Kruskal-Wallis test was used, with Bonferroni-corrected pairwise comparisons performed if significant differences were detected. Statistical significance was set at $p < 0.05$.

Results

Antioxidant Effects of Carvacrol on Liver Damage Induced by ATO: A Study of Alkaline Phosphatase (ALP) and LDH Activities

Figure 1 shows that ATO treatment (5 mg/kg/day) induced significant liver injury in rats, as evidenced by increased serum ALP activity compared to the control group ($P < 0.05$). Similarly, LDH activity was significantly elevated in the ATO–treated group ($P < 0.05$). In contrast, administration of carvacrol at doses of 50, 100, and 200 mg/kg/day significantly ameliorated these enzyme activities ($P < 0.05$).



A B

Figure 1. Improving effect of carvacrol on serum ALP and LDH enzyme activities. The results are presented as mean ± standard deviation for ten animals per group. * Indicates a significant difference compared to the control group ($P < 0.05$), while # indicates a significant difference compared to the ATO group ($P < 0.05$).

Antioxidant Effects of Carvacrol on Liver Damage Induced by ATO, ALT, and AST Activity

As shown in Figure 2, ATO administration (5 mg/kg/day) significantly increased serum AST activity compared to the control group ($P < 0.05$). Similarly, ALT activity was markedly elevated in ATO–treated animals ($P < 0.05$). Conversely, carvacrol administration at 50, 100, and 200 mg/kg/day significantly increased these enzyme levels.

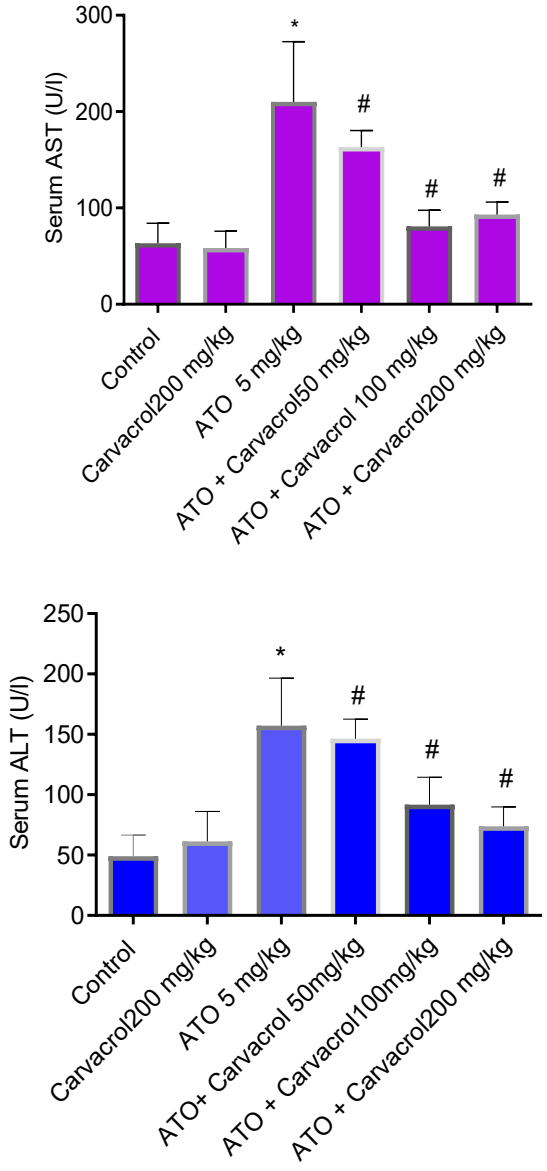


Figure 2. Improving effect of carvacrol on serum AST and ALT enzyme activities. The results are presented as mean ± standard deviation for ten animals per group. * Indicates a significant difference compared to the control group ($P < 0.05$), while # indicates a significant difference compared to the ATO group ($P < 0.05$).

Antioxidant Effects of Carvacrol on Liver Damage Induced by ATO: A Study of Oxidative Stress Index and ROS

As indicated in Figure 3, ATO administration (5 mg/kg/day) significantly increased ROS and oxidative stress index levels in the treated animals compared to the control group ($P < 0.05$). Additionally, the same dose significantly reduced the antioxidant capacity relative to controls ($P < 0.05$). In contrast, carvacrol treatment at 50, 100, and 200 mg/kg/day markedly improved these parameters ($P < 0.05$).

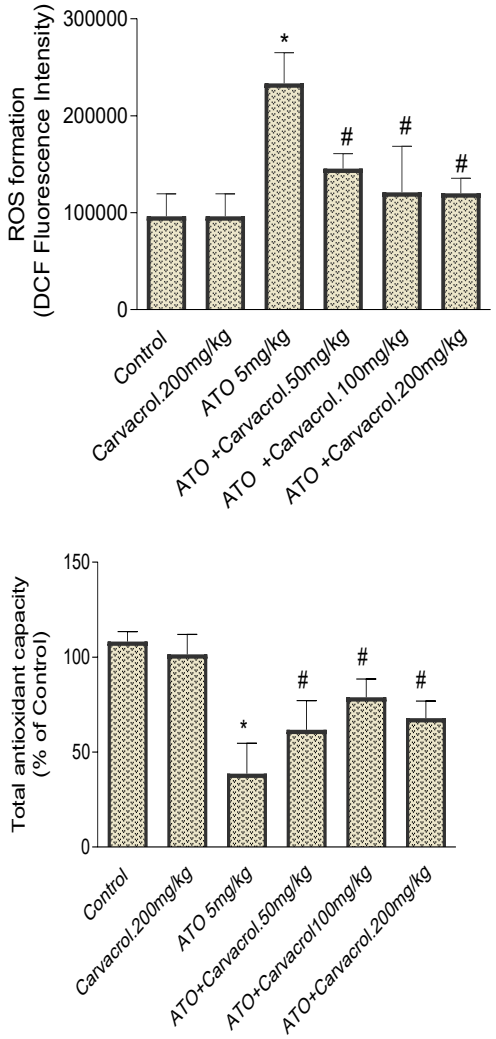
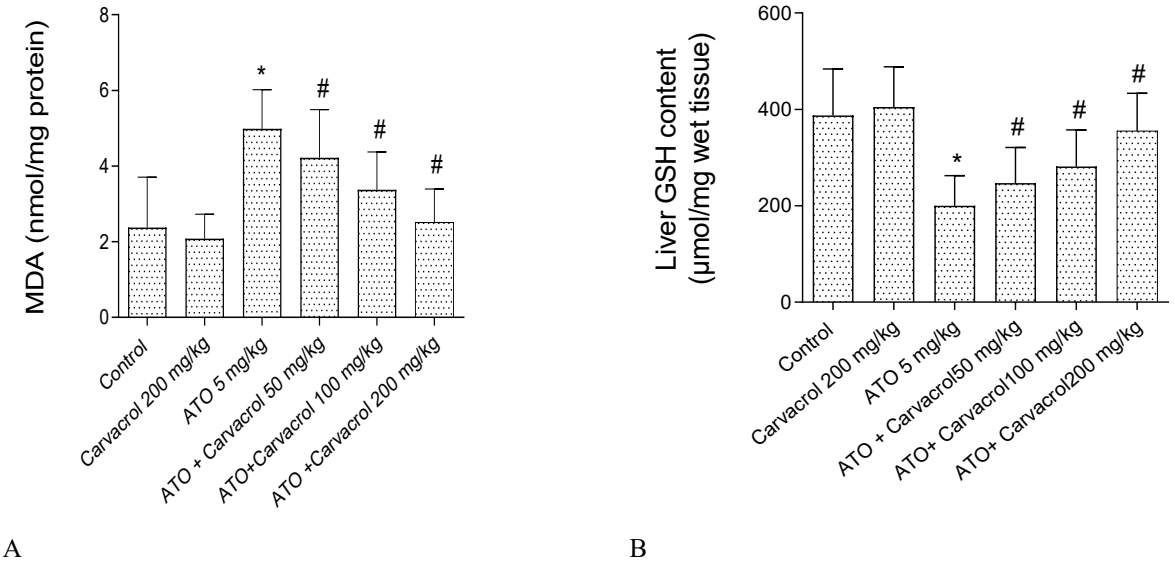


Figure 3. Improving effect of carvacrol on serum ROS and oxidative stress index enzyme activities. The results are presented as mean ± standard deviation for ten animals per group. * Indicates a significant difference compared to the control group ($P < 0.05$), while # indicates a significant difference compared to the ATO group ($P < 0.05$).

Antioxidant Effects of Carvacrol on Liver Damage Induced by ATO: A Study of Lipid Peroxidation, MDA, and GSH Levels

As demonstrated in Figure 4, ATO administration (5 mg/kg/day) significantly increased MDA levels in treated animals compared to the control group ($P < 0.05$). Additionally, the same treatment markedly reduced GSH levels ($P < 0.05$). In contrast, carvacrol administration at 50, 100, and 200 mg/kg/day significantly increased both MDA and GSH levels ($P < 0.05$).



A B

Figure 4. Improving effect of carvacrol on serum MDA and GSH enzyme activities. The results are presented as mean ± standard deviation for ten animals per group. * Indicates a significant difference compared to the control group ($P < 0.05$), while # indicates a significant difference compared to the ATO group ($P < 0.05$).

Discussion

In recent years, ATO has been approved for the treatment of APL and is widely utilized in Iran. Although highly effective, ATO can cause side effects including fluid retention, leukocytosis, gastrointestinal disturbances, fatigue, neuropathy, and hepatotoxicity. A primary concern is hepatotoxicity induced by ATO–mediated oxidative stress. Carvacrol demonstrates hepatoprotective effects by mitigating oxidative stress and inflammation in experimental models of liver injury [19]. Our study involved administering ATO to mice at 5 mg/kg, either alone or in combination with varying doses of carvacrol. We evaluated liver injury biomarkers (ALT, AST, LDH, and bilirubin), histopathological changes, and oxidative stress indicators, including lipid peroxidation, ROS, GSH content, and antioxidant capacity. The results demonstrated that ATO induces liver damage, as evidenced by increased liver injury biomarkers (ALT, AST, LDH, and bilirubin), elevated lipid peroxidation and ROS levels, and reduced GSH content and antioxidant capacity. However, carvacrol effectively mitigated liver damage, providing partial hepatoprotective effects. These findings are consistent with the hypotheses of our study. In the following section, relevant literature is reviewed.

Consistent with our results, Zhang et al. (2013) reported that administration of ATO (3 mg/kg) significantly reduced superoxide dismutase and catalase activities, and altered the GSH/GSSG ratio in rats. Resveratrol was indicated to enhance antioxidant enzyme activity, improve liver histopathology, and reduce arsenic accumulation and its associated oxidative stress. Intake of potent antioxidants may help mitigate this hepatotoxicity [20].

Studies from 2020 and 2022 demonstrated that ATO–induced hepatotoxicity is associated with elevated liver injury markers (AST, ALT), increased lipid peroxidation (MDA), and decreased levels of antioxidant enzymes, including catalase, GSH peroxidase, and superoxide dismutase. Arsenic-induced disruption of hepatocyte mitochondrial function promotes oxidative stress and liver injury, whereas antioxidant compounds can confer protective effects. These findings are consistent with the results of the present study. In recent years, consumption of fruits and vegetables has increased owing to their protective effects against diseases, including cancer, cardiovascular disorders, and liver diseases [21, 22]. Carvacrol, a monoterpenoid phenol abundantly found in essential oils of plants, such as thyme and oregano, exhibits strong antioxidant, anti-inflammatory, and hepatoprotective properties. Preclinical studies summarized in Carvacrol's review in 2024 indicate that it neutralizes free radicals, inhibits lipid peroxidation, and restores endogenous antioxidant defenses, including enhancement of catalase, superoxide dismutase, GSH peroxidase, and GSH levels. These effects mitigate oxidative stress–induced cellular damage, preserve membrane integrity, and protect liver tissue from toxic insults. Given these multifaceted protective mechanisms, Carvacrol emerges as a promising natural compound to attenuate chemically induced hepatotoxicity and support liver health [23]. A study reported that administration of 500 mg/kg of silver nanoparticles decreased total antioxidant capacity (TAC), increased lipid peroxidation, elevated ALT and AST levels, and induced histopathological changes in the liver. Conversely, carvacrol administered at 100 mg/kg effectively mitigated these damages and significantly improved both biochemical and histopathological parameters [24]. These findings are consistent with the present study and underscore the beneficial effects of carvacrol on

biochemical markers and oxidative stress. The study demonstrated that ketamine administration induced oxidative stress in testicular tissues, evidenced by increased MDA levels and histopathologic damage. Treatment with 50 mg/kg carvacrol significantly reduced MDA levels, enhanced antioxidant enzyme activity, and mitigated the histopathologic alterations caused by ketamine [25]. These findings support the beneficial effects of thyme, rich in carvacrol, on biochemical markers and oxidative stress, consistent with the results of the present study.

Conclusions

The present investigation revealed that, however, carvacrol can dose-dependently reduce oxidative enzyme levels, thereby mitigating liver toxicity induced by ATO. Nevertheless, further research is needed to elucidate its precise mechanism of action. One of the limitations of our study is the lack of investigation into the signaling pathways, and it is suggested that future studies focus on a more detailed examination of these pathways and the molecular interactions of carvacrol with them to gain deeper insights into its mode of action. Additionally, it is recommended that researchers investigate potential pharmacodynamic and pharmacokinetic interactions between carvacrol and ATO in future studies to ensure that the combination of these two compounds does not result in negative effects.

Conflicts of Interest

The authors declare no competing interests.

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Compliance with Ethical Guidelines

All experimental procedures involving animals were performed in accordance with institutional and national guidelines for the care and use of laboratory animals. The study protocol was reviewed and approved by the Ethics Committee of Lorestan University of Medical Sciences, Iran (Ethical approval code: IR.LUMS.REC.1402.186). Every effort was made to minimize animal suffering and to reduce the number of animals used.

Authors' Contributions

Conceptualization: Hamidreza Mohammadi, Amin Hasanvand

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Methodology: Mahnoush Askarifard Jahromi, Ahmad Adineh

Experimental work/Data collection: Amin Hasanvand, Mohsen Mohammadi, Mehrdad Gholami, Saeedeh Ahmadipour

Data analysis and interpretation: Amin Hasanvand, Ahmad Adineh

Manuscript drafting: Amin Hasanvand, Mohsen Mohammadi

Critical revision of the manuscript: Hamidreza Mohammadi

Supervision: Hamidreza Mohammadi

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