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Research Paper

Protective Effect of Carvacrol Against Acute Liver Damage and Encephalopathy Induced by Thioacetamide

Anahita Esmaeili[1], Peyman Astaraki[2], Mohammad Jamalian[3], Hamid Reza Mohammadi[4*], Naser Reza Ziyaeenia[5]

[1] *Pharmacy Student, Student Research Committee, Lorestan University of Medical Sciences. Khoram-Abad, Iran.*
[2] *Department of Internal Medicine, School of Medicine, Shahid Rahimi Hospital, Lorestan University of Medical Sciences, Khoram-Abad, Iran.*
[3] *Department of Forensic Medicine, School of Medicine, Valiasr Hospital, Arak University of Medical Sciences, Arak, Iran.*
[4] *Department of Toxicology, Faculty of Pharmacy, Razi Herbal Medicine Research Center, Lorestan University of Medical Sciences, Khoram-Abad, Iran.*
[5] *Department of Health Education, School of Medicine, Lorestan University of Medical Sciences, Khoram-Abad, Iran*

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*** Corresponding author:**
Esmaeili A, Department of Toxicology, Faculty of Pharmacy, Razi Herbal Medicine Research Center, Lorestan University of Medical Sciences, Khoram-Abad, Iran.
E- mail: hamidrezamohammadi65@yahoo.com

ABSTRACT

Background: Cancer occurs in 83% of liver diseases. Other risk factors for liver cancer include viral hepatitis, alcohol consumption, industrial chemicals, and a number of toxins. Another major disease that occurs following liver damage is hepatic encephalopathy. This condition arises primarily due to increased blood ammonia levels. Carvacrol, with antioxidant properties, reduces oxidative stress on the liver. The aim of this study was to investigate the effect of carvacrol on the improvement of hepatic encephalopathy in rats.

Methods: In this experimental study, 60 male Wistar rats were randomly divided into six groups of 10 each. Liver damage and induction of oxidative stress were caused in the rats by administering thioacetamide (100 mg/kg/day) intraperitoneally for three consecutive days. Carvacrol was administered by gavage at 25, 50, or 100 mg/kg/day after thioacetamide treatment. We investigated the biomarkers of liver damage in the blood, such as alanine transaminase, lactate dehydrogenase, total protein, and bilirubin. We also assessed the effect of oxidative stress, as the key inducer of hepatic encephalopathy, on the liver by measuring the lipid peroxidation, antioxidants, reactive oxygen species, glutathione reserves, and ammonium levels in the serum and brain.

Results: Thioacetamide significantly increased the biochemical markers in the rat sera, reflecting ammonium release and the development of oxidative stress ($P<0.05$). Conversely, the various doses of carvacrol significantly reduced the levels of biomarkers that are indicative of liver damage ($P<0.05$).

Conclusion: The study findings provided experimental evidence in favor of the therapeutic effects of carvacrol and against liver injury induced by thioacetamide, leading to encephalopathy.

Keywords: Ammonium, Carvacrol, Hepatic encephalopathy, Oxidative stress, Thioacetamide liver damage

Introduction

Liver cancer is the fifth most common disease worldwide and arises in about 83% of people with serious liver diseases. Viral hepatitis, food additives, alcohol, fungal toxins, industrial chemicals, and air and water pollutants account for some of the liver cancer risk factors [1]. Thioacetamide is one of the poisonous compounds, which is widely used in various industries. It comes as a colorless powder with a mild mercaptan smell. This compound is soluble in water mixed with ethanol and slightly soluble in water alone. When heated up to decomposition, it releases toxic gases, containing nitrogen and sulfur oxides. Thioacetamide also has the ability to destroy liver cells and cause cirrhosis. This substance is toxic to the liver and affects the synthesis of DNA, RNA, protein, and glutathione, leading to pathologic alterations in the liver [2].

Plants and their derivatives have been considered sources of therapeutic compounds for decades. In recent years, there has been much interest in applying herbal compounds to the treatment of various diseases, mainly because of their lower side effects than chemical drugs [3]. Carvacrol is a phenolic monoterpenoid compound with antimicrobial, antioxidant, and anticancer properties [4]. It is found in numerous plants, such as oregano, thyme, bell pepper, mint, wild bergamot, and others [4]. Studies have shown that carvacrol can neutralize free radicals effectively [5, 6]. The antioxidant and antimicrobial activities of carvacrol are due to the presence of free hydroxyl groups and their hydrophobicity [7].

Further, carvacrol has bacteriostatic effects, preventing bacterial growth, and also bactericidal properties against most species, including *Salmonella typhimurium* and *Escherichia coli* [8]. It also exhibits antiproliferative effects against various neoplasias, including prostate, liver, lung, and cervical cancers, as well as leukemia [9]. All essential oils containing carvacrol are believed to have antifungal, antiviral, and antimicrobial effects [10]. To a large extent, carvacrol improves glutathione synthesis by the liver, kidneys, and spleen while reducing the blood malondialdehyde level [11].

Aim of the Study: Despite the various therapeutic benefits associated with carvacrol, neither the beneficial nor adverse effects have been fully investigated to date. Therefore, this study was conducted to investigate the beneficial effects of carvacrol on the liver of rats damaged by thioacetamide administration.

Materials and Methods

This experimental study was conducted at the Faculty of Pharmacy, Lorestan University of Medical Sciences, Khoram-Abad, Iran, in 2023. All ethical principles that were observed in this study were based on the international guidelines for the use of laboratory animals. The experimental protocol was also reviewed and approved by the Ethics Committee of Lorestan University of Medical Sciences (Code: IR.LUMS.REC.1402.185).

Animal Grouping: To conduct this study, 60 male Wistar rats weighing 220±20 g were randomly divided into six groups of 10 each as follows:

1. Group 1 (zero control) received only normal saline.
2. Group 2 received only thioacetamide at 100 mg/kg/day for three days.
3. Group 3 received thioacetamide (100 mg/kg/day x3 days) + carvacrol (10 mg/kg/day x3 days).
4. Group 4 received thioacetamide (100 mg/kg/day x3 days) + carvacrol (25 mg/kg/day x3 days).
5. Group 5 received thioacetamide (100 mg/kg/day x3 days) + carvacrol (50 mg/kg/day x3 days).
6. Group 6 received the highest dose of carvacrol at 50 mg/kg/day x3 days.

Ethical Guidelines: All drug doses were selected based on the criterion of being harmless to the study animals. This study was conducted according to the ethical guidelines set and approved by the Ethics Committee of Khoram-Abad University of Medical Sciences, Khoram-Abad, Iran.

Liver Damage Induction: In order to induce damage and acute injury to the liver in the animals, thioacetamide was administered to the rats at a dose of 100 mg/kg by intraperitoneal (IP) injection every 24 hours for three consecutive days [12, 13]. Then, 24 hours after the last thioacetamide dose, the specific experiment was performed on each group of animals. In order to prevent hypoglycemia and death of the animals by the liver damage model caused by thioacetamide, the drinking water of the rats contained 1% dextrose (w/v) throughout the study. Varying doses of carvacrol (10, 25, or 50 mg/kg) were administered to the rats by gavage daily for seven continuous days. Routinely, each carvacrol dose was given orally one hour after the thioacetamide injection. Carvacrol was purchased from Sigma-Aldrich (St. Gallen, Switzerland).

Biochemical Parameters: At the end of the experimental period, blood samples were collected by puncturing the heart of each sacrificed rat. The blood samples were immediately transferred to the laboratory and centrifuged at 4,000 rpm for 10 min. The serum levels of liver enzymes (alanine transaminase [ALT] and lactate dehydrogenase), total protein, and bilirubin were measured using an autoanalyzer (Selectra Pro M; Tehran, Iran) based on the instructions provided by the Pars Azmoun enzyme kits (Tehran, Iran).

Motor Activity Assessment: This part of the study, which was called the open field test, was planned to investigate the effects of incremental serum ammonia levels on the behavior and locomotion of the rats. On this test, the activity and motor coordination of the rats were assessed between the first and the fourth days every 24 hours [12]. The test was performed in such a way that the animal was placed in a white cubic box (100 × 100 × 30 cm). The box floor was divided into small squares of 20 × 20 cm. Before the experiment, each rat was placed in the cubic box for one minute to get used to the test environment. Then, each rat was placed on the central square, and the number of squares that each rat walked on or crossed was counted, as described in an earlier study [12].

Markers of Liver Oxidative Stress

Lipid Peroxidation Assay: The tissue malondialdehyde (MDA) level was measured by the Ohkawa method [14]. This method validly evaluates the ability of MDA to interact with thiobarbituric acid under acidic conditions, which leads to the production of pink color in the reaction mixture. Briefly, 0.5 ml of 10% homogenous mixture was added to a test tube to which 3 ml of 1% phosphoric acid and 1 ml of 0.6% thiobarbituric acid were added. The mixture was then heated at 100ºC for 45 min. After cooling, 4 ml of n-butanol was added to the mixture, which was then thoroughly agitated.

Glutathione Level: Glutathione exists in two forms: oxidized (GSSG) and reduced (GSH) in different tissues. GSSG is converted to GSH by the enzyme glutathione reductase. The substance 5,5′-Dithiobis-(2-nitrobenzoic acid; DTNB) is known to identify thiol-containing groups. For this purpose, 200 mg of each rat's liver tissue was homogenized and suspended in 8 ml of cold EDTA solution at 0.02 M. Following that, a 5-ml homogenized liver mixture was added to 4-ml water and 1-ml trichloroacetic acid (50% w/v) and

stirred well. This mixture was then centrifuged at 3,000 rpm for 15 min, and 2 ml of the resultant supernatant was removed and mixed with 4-ml Tris buffer (0.4 M) and 0.1-ml DTNB (0.01 M). The test tube was then shaken well, and its absorbance was read at 412 nm [15].

Generation of Reactive Oxygen Species: To determine the level of reactive oxygen species (ROS) in each sample, 500 mg of the liver tissue from each rat was added to 5-ml of Tris-hydrochloride buffer (40 mM) at pH 7.4 (4ºC), and homogenized. A 100 µl of the homogenous mixture was added to 1-ml Tris-hydrochloride buffer (40 mM, pH 7.4) and 2′,7′-dichlorofluorescein diacetate at 1-µM concentration. These samples were then incubated at 37ºC for 15 min in the dark. Finally, the fluorescence intensity of each sample was measured at 485 nm and 525 nm (i.e., the excitation and emission wavelengths, respectively) using a fluorimetry spectroscopy unit [16].

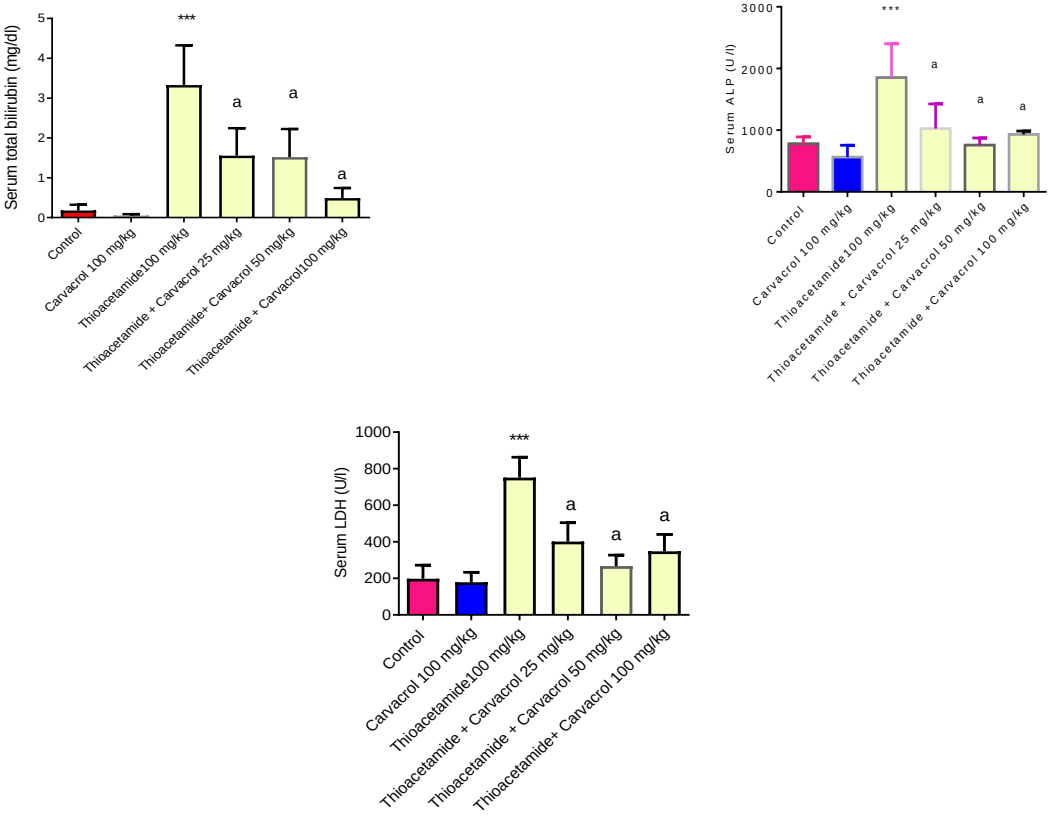
Total Antioxidant Capacity: A 100-µl homogenous mixture from each liver tissue sample was added to 3-ml ferric reducing ability of plasma solution (containing 2.5 ml acetate buffer; at 300-mM, pH 3.0), and then 0.25 ml of iron chloride solution (20-mM) and 0.25-ml ferric 2,4,6-tripyridyl-s-triazine solution were added and incubated at room temperature for 5 min. After centrifugation at 10,000 G for 1 min, the absorbance of each sample was read at 593 nm using a spectrophotometer. Finally, the data obtained from the spectrophotometry were constructed as standard plots, and the total antioxidant capacity was calculated for each sample. This provided the µl equivalent of vitamin C [17].

Statistical Analyses: The data were presented as the means and standard deviations. The obtained data were analyzed using a one-way ANOVA test. Tukey's supplementary test was used to check for the statistical differences among the animal groups.

Results

Liver Enzymes: Comparing the results of the statistical tests associated with the activity levels of ALT, aspartate transaminase, and alkaline phosphatase enzymes showed that the thioacetamide treatment of animals led to significant increases in the activities of liver enzymes compared to that of the control group (P<0.001). Conversely, the carvacrol treatment in the groups receiving thioacetamide prevented rises in the activities of liver enzymes compared to that of the group that received thioacetamide only (P<0.001).

Serum Bilirubin and Protein Levels: The data representing the total serum bilirubin and protein concentrations among the group receiving only thioacetamide compared to the control group exhibited significant increases in the bilirubin and total protein levels (P<0.001). In groups that received the various doses of carvacrol, the levels of bilirubin and protein were significantly lower than that of the group that received thioacetamide only (P<0.001). See Figure 1.



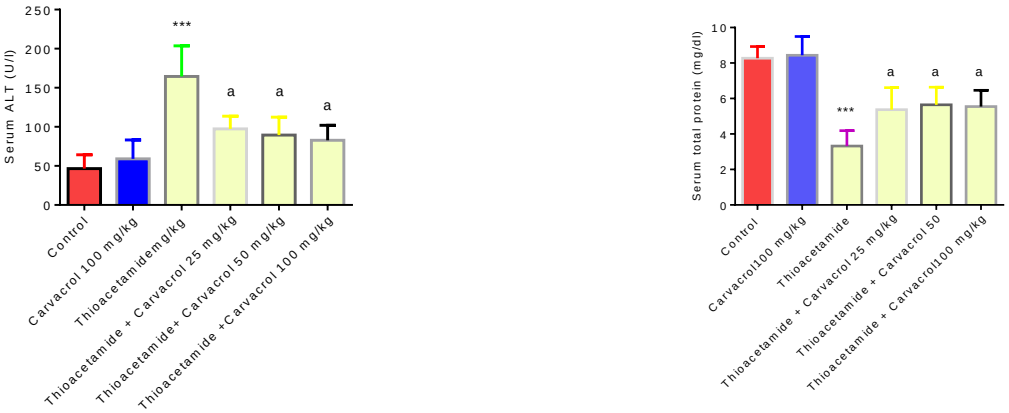


Figure 1: Effects of treatment with thioacetamide and carvacrol on the biochemical indices of liver damage in rats between the treatment and control groups (n=10 mice/group).
*** Denotes a statistical difference between the group receiving thioacetamide and the control group (P<0.001).
a = Shows a statistical difference between the group receiving carvacrol and the group receiving thioacetamide (P<0.001).

Serum Ammonium Levels: Treating rats with thioacetamide significantly increased the serum levels of ammonium compared to that of the control group (P<0.001). However, the administration of carvacrol in the groups receiving thioacetamide prevented the rise in the serum ammonium levels compared to that of the group that received thioacetamide only (P<0.001 and P<0.05). See Figure 2.

Animal Locomotion: Comparing the data for the animal locomotion indicated that the total number of movements or locomotion of the animals undergoing thioacetamide treatment was much lower than that of the control group (P<0.001). In the rat groups that received carvacrol, the motor activities showed high improvements (P<0.001). See Figure 3.

Lipid Peroxidation and ROS Activities: The serum levels of lipid peroxidation and ROS activities significantly increased in the rats treated with thioacetamide compared to that of the control group (P<0.001). Furthermore, the levels of thiobarbituric acid reactive substances (TBARS) and ROS in the treated rats decreased after carvacrol treatment compared to the rat group that received thioacetamide treatment only (P<0.001). See Figure 4.

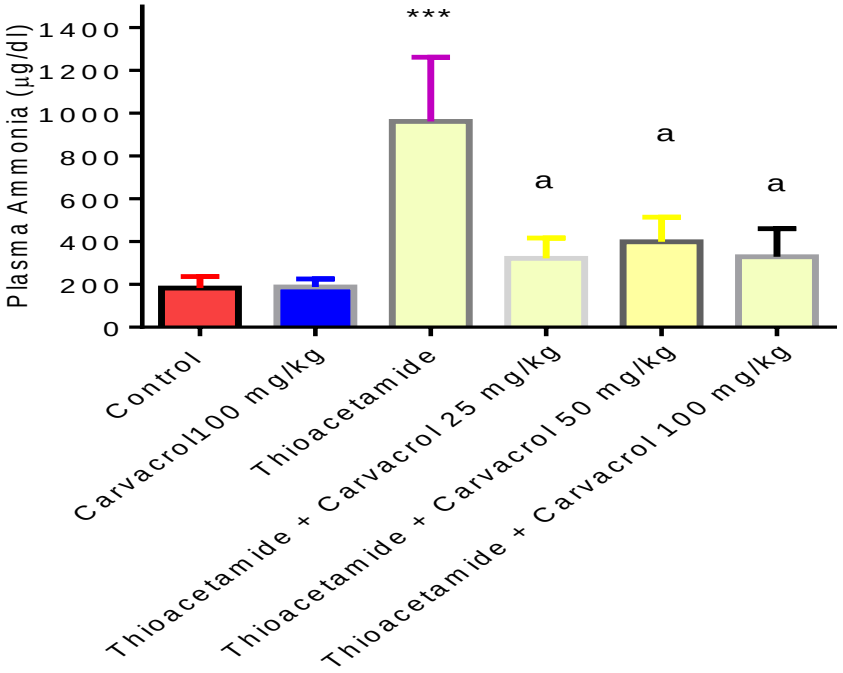


Figure 2: Effects of treatment with thioacetamide and carvacrol on the plasma level of ammonium ions between treatment and control groups (n=10 mice/group).
*** = Indicates a statistical difference between the group receiving thioacetamide and the control group (P<0.001). **a** = Denotes a statistical difference between the group receiving carvacrol and the group receiving thioacetamide (P<0.001).

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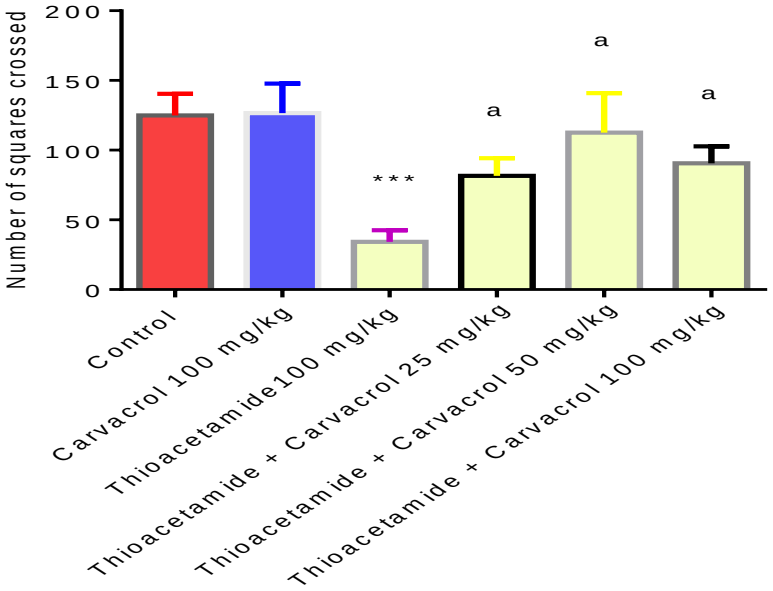

Figure 3: Effects of treatment with thioacetamide and carvacrol on the movement activity of animals between treatment and control groups (n=10 mice/group). *** = Shows a statistical difference between the group receiving thioacetamide and the control group (P<0.001). **a** = Shows a statistical difference between the group receiving carvacrol and the group receiving thioacetamide (P<0.001).

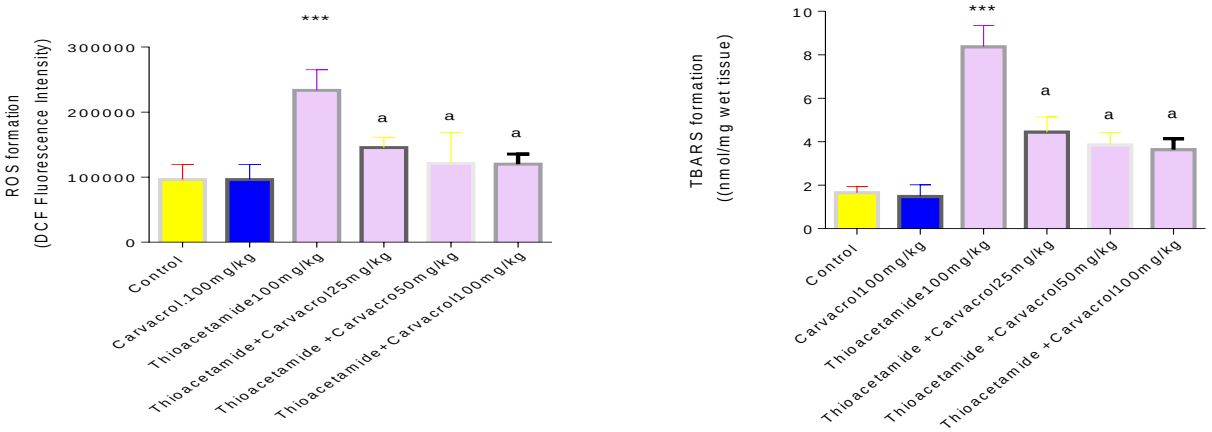

Figure 4: Effects of treatment with thioacetamide and carvacrol on oxidative stress indicators (lipid peroxidation and reactive oxygen species) of animals between treatment and control groups (n=10 mice/group). *** = Shows a statistical difference between the group receiving thioacetamide and the control group (P<0.001). **a** = Shows a statistical difference between the group receiving carvacrol and the group receiving thioacetamide (P<0.001).

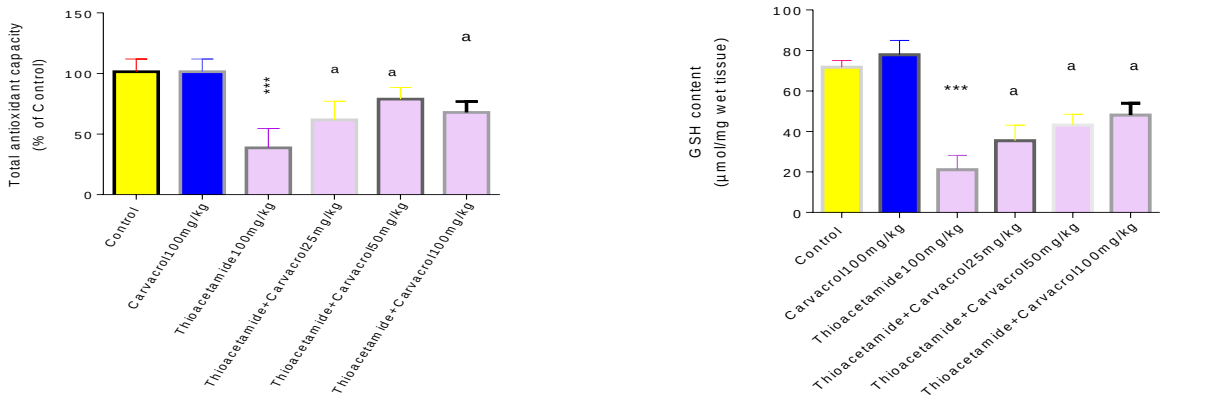

Figure 5: Effects of treatment with thioacetamide and carvacrol on oxidative stress indicators (glutathione and antioxidant capacity) between treatment and control groups (n=10 mice/group). *** = Shows a statistical difference between the group receiving thioacetamide and the control group (P<0.001). **a** = Shows a statistical difference between the group receiving carvacrol and the group receiving thioacetamide (P<0.001).

Glutathione and Antioxidant Activities: The glutathione (GSH) and antioxidant activities significantly decreased in the rats treated with thioacetamide only compared to those found in the control group (P<0.001). However, the carvacrol treatment in the groups receiving thioacetamide prevented the reductions in the GSH and antioxidant activities of the rats compared to those that received carvacrol only (P<0.001). See Figure 5.

Discussion

This study was conducted to investigate the effects of carvacrol treatment on rat liver damage induced by acute liver failure experimentally and the key factor causing hepatic encephalopathy, which rises in the blood ammonium level resulting from treatment with thioacetamide. Acute liver failure can occur due to various causes, such as viral infections, drugs, and the effects of various toxins [18]. Regardless of the cause, acute liver damage and the subsequent encephalopathy can lead to death if not treated. On the other hand, there is no safe and specific drug treatment for the prevention or treatment of acute liver failure and subsequent problems, such as brain injury.

The findings of the current study provided evidence that carvacrol treatment increased the antioxidant defense of the liver and naturally reduced lipid peroxidation. The study results showed that the thioacetamide treatment induced oxidative stress in the rats' liver. Conversely, carvacrol acted as an antioxidant compound against the significant oxidative stress in the rats' liver induced by thioacetamide. The results of the current research are consistent with those reported by previous studies [19, 20].

Thioacetamide consumption in rats significantly increased the concentrations of TBARS as valid indices of lipid peroxidation compared to that of the control group. In this regard, treating the animals with carvacrol effectively reduced the elevated TBARS levels, bringing them close to those observed in the control group. The results of lipid peroxidation in this study were essentially the same as those reported by a previous work on the oxidative stress caused by thioacetamide in rat kidneys [21].

It is believed that carvacrol plays a significant role in maintaining cell membrane stability and function. Previous studies have shown that carvacrol maintains the balance between ethanolamine and phosphatidylcholine by participating in the methylation processes inside the cell membrane [22, 23]. Therefore, carvacrol protects the cell membrane functions and prevents abnormal lipid peroxidation. Animal studies have demonstrated that a number of antioxidants prevent oxidative damage through a series of cellular mechanisms that control oxidation in the body [24, 25]. The glutathione antioxidant system also plays a fundamental role in defending the cell membranes against ROS. In addition, superoxide dismutase protects cells from damage caused by free radicals resulting from peroxidation [26].

In the current study, while the serum levels indicative of liver damage increased, we found that the antioxidant capacity, glutathione storage, and the oxidized glutathione ratio to GSH in the liver also decreased significantly. Conversely, a significant amount of ROS increased in oxidized glutathione reserves, and rises in lipid peroxidation and protein oxidation were found in the damaged liver tissue samples of the rats. All of the observations of the current study were consistent with those reported by previous research [27, 28]. Equally important, the former studies did not demonstrate that oxidative stress played a major role in causing liver damage.

In a study conducted in 2016 [29], the protective effects of carvacrol against oxidative damage in rat liver were investigated. That research showed that the extent of liver damage in 20-month-old rats was higher than those in 10-month-old rats, with the level of lipid peroxidation being also higher. On the other hand, the results of the mentioned study suggested that carvacrol inhibited oxidative stress and improved the antioxidant defense. Hence, carvacrol was indirectly recommended as a therapeutic agent for inhibiting the development or progression of liver damage due to aging [29].

In another study conducted in 2022 [30], it was reported that carvacrol significantly increased the activities of catalase, superoxide dismutase, and glutathione peroxidase, which are three well-known antioxidant enzymes. These enzymes also reduce or inhibit the activities of *Candida auris*, which causes fungal infections [31]. The results of that research [31] are consistent with those of the current study. The beneficial effects of carvacrol on liver tissue have been shown in other studies as well [29, 32]. Several mechanisms have been proposed for the cytoprotective effects of carvacrol. A major mechanism is the antioxidant activity of carvacrol, which has been reported by earlier studies [24, 33]. Carvacrol reduces oxidative stress and protects against damage to biological membranes and cellular proteins [25]. In the current study, it was found that different doses of carvacrol effectively prevented oxidative stress and the subsequent damage to the rats' liver tissue. Therefore, a large part of the protective effects of carvacrol in rats treated with thioacetamide may be applied through its inhibition of oxidative stressors and scavenging free radicals, such as ROS. The pivotal argument here is that free radicals are well-known to cause damage to the DNA strands [34] and make detrimental alterations in the cytoskeleton [35].

Conclusions

The present study provides evidence that carvacrol offers protective and antioxidant effects by inhibiting oxidative stress that leads to harmful impacts on the rat liver. However, further investigations are warranted to

evaluate the key liver enzymes and functions in animal models. Such strategies can accurately and specifically determine the beneficial effects of carvacrol in preventing the adverse side effects of thioacetamide on the liver in animal models and potentially in humans.

Conflict of Interests

The authors have no conflicts of interest to declare in conducting this research.

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Compliance with Ethical Guidelines

The present study was reviewed and approved by the Committee on Animals Rights of Lorestan University of Medical Sciences (Approval code: IR.LUMS.REC. 1402.185).

Authors' Contributions

The authors contributed fairly equally in proposing the research concept, study design, and protocol, conducting the experiments, analyzing the data, and writing several drafts of the manuscript. All authors reviewed and approved the final version of the manuscript prior to submission to this journal for publication.

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