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Investigation of the Protective Effects of Carvacrol on Cholemic Nephropathy in Cholestatic Rats

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ABSTRACT

Background and Objective: Kidney is one of the organs that is damaged after liver cirrhosis. This damage to kidney tissue following liver tissue failure is called cholemic nephropathy. Since one of the main mechanisms in the development of cholemic nephropathy is oxidative stress and reduced antioxidant reserves, the present study was conducted to investigate the protective effect of carvacrol as an antioxidant on cholemic nephropathy in cholestatic rats.

Methods: This experimental study was conducted on 40 male Wistar rats in 5 groups of 8. Cholestasis was induced by bile duct ligation (BDL). Animals received different doses of carvacrol (25, 50, 100 mg/kg) via gavage. At the end of the period and after carvacrol administration, serum biochemical factors such as creatinine (Cr) and blood urea nitrogen (BUN) and urinary factors including glucose and protein, levels of reactive oxygen species (ROS), lipid peroxidation (MDA) as well as the activities of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) were evaluated in kidney tissue of cholestatic rats.

Findings: Serum levels of BUN and Cr in cholestatic rats were (59±2, 0.8), respectively, which were increased compared to the control groups (17±2, 0.18) (p=0.002). Serum levels of oxidative stress indicators MAD, ROS in cholestatic rats were (17±1, 154109), respectively, which were significantly higher than the control groups (5±1, 37124±11) (p=0.007). Moreover, the activities of antioxidant enzymes (CAT, SOD, GPx) in cholestatic rats were (200±11, 19±3, 20±1), respectively, which were significantly decreased compared to the control groups (600±34, 64±2, 43±1) (p=0.001).

Conclusion: The results of the study showed that administering carvacrol can reduce oxidative stress caused by bile duct ligation in the kidneys.

Keywords: *Oxidative Stress, Bile Duct Ligation, Cholemic Nephropathy, Carvacrol*.

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Introduction

Kidney injury is a common complication in patients with liver disease, especially those with chronic liver disease such as cirrhosis (1, 2). Although the exact mechanism for how this complication occurs has not yet been determined, previous studies have emphasized the major role of bile acids in the development of this problem (2-4). This complication in patients with cirrhosis is known as cholemic nephropathy (5). Acute kidney injury (AKI) is an important complication in patients with liver disease and jaundice, as it is associated with significant morbidity and mortality. Cholemic nephropathy (CN) is thought to be an important cause of AKI in advanced liver disease with jaundice (6). Cholemic nephropathy is damage to kidney tissue caused by bile duct obstruction (7).

Liver failure, especially in chronic conditions such as cirrhosis, causes an excessive increase in serum bile acids. Since bile acids are excreted mainly by the kidneys during liver failure and bile duct damage, these substances can cause serious damage to kidney tissue and complete failure of this organ (4, 5). Normally, bile acids are secreted into the small intestine through the bile ducts and exert their physiological effects. Bile acids, especially the hydrophobic types of these substances, have detergent properties and cause emulsification of fat particles entering the intestines. They also have potentially destructive effects on membranes. These substances can damage biological membranes and destroy their lipid structure (3, 8). Since one of the main mechanisms of kidney injury as a result of bile acid accumulation in the liver is the induction of oxidative stress and reduction of antioxidant reserves in cells, and this has been abundantly mentioned in previous studies (9, 10), natural antioxidants are needed to reduce this damage. Given that chemical drugs used to treat kidney problems have side effects, the use of products with natural origin with fewer side effects is emphasized (11).

Carvacrol is a monoterpenoid phenol that is a component of various essential oils and is usually found in abundance with its isomer thymol in thyme, marjoram, black walnut, bergamot, and tomato plants (12). Today, the edible extract of thyme, which contains carvacrol, is widely used as an antitussive and even antioxidant substance (13). Carvacrol is used as a food additive (14). Numerous studies have shown that carvacrol has anti-inflammatory (15), antimicrobial (16), antithrombotic (17), antispasmodic (18), vasodilator (19), and antifungal (20) activities. One of the properties of carvacrol that has recently attracted the attention of researchers is its antioxidant properties. Carvacrol, as a strong antioxidant, has free radical scavenging activity. Findings from studies have shown that carvacrol consumption reduces liver cell death following induction of hepatic ischemia-reperfusion, and that the reduction in cell death is related to the inhibition of oxidative stress and the prevention of free radical accumulation (21). Given that carvacrol is a potent antioxidant and oxidative stress is one of the main causes of damage in colic nephropathy, the aim of this study was to investigate the effect of different doses of carvacrol on the inhibition of oxidative stress and biochemical changes in the renal complication of colic nephropathy caused by bile duct ligation in a rat model.

Methods

This experimental study was conducted after approval by the Ethics Committee of Lorestan University of Medical Sciences with the ethics code IR.LUMS.REC.1402.061. Carvacrol was purchased from Sigma Corporation. Special kits for measuring antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase) and measuring blood and urine samples (protein, glucose, urea and creatinine) were obtained from ZIST SHIMI Company. Male Wistar rats, aged 6-7 months and weighing 180-220 grams, were

obtained in 2023 and were housed in the animal house of the Faculty of Pharmacy of Lorestan University of Medical Sciences under standard controlled conditions at a temperature of 20-25°C and a 12-hour light/dark cycle. All rats had free access to drinking water and laboratory food and were kept under identical conditions (22).

In this study, 40 male Wistar rats were randomly divided into 5 groups of 8 as follows: The first group underwent only surgery but the bile duct was not ligated and was considered as a healthy control group (Sham). The second, third, fourth and fifth groups underwent surgery and their bile duct was completely ligated. Then, for 14 days, only normal saline was administered to the second group, and the third, fourth and fifth groups were administered orally once a day with a carvacrol solution at a concentration of 25, 50 and 100 mg/kg/day, respectively. After 14 days, the rats were anesthetized by intraperitoneal injection of 10 mg/kg xylazine and 70 mg/kg ketamine, and blood samples were collected from the animal's heart. Blood urea nitrogen (BUN) and creatinine (Cr) levels in blood samples and protein and glucose levels in urine samples were measured by a special kit to ensure the occurrence of cholemic nephropathy in rats in second, third, fourth and fifth groups. Finally, the animals were sacrificed and their kidney samples were used to examine oxidative stress index and antioxidant enzymes (23).

Biochemical tests: Blood and urine samples were centrifuged immediately after collection and the obtained plasma was frozen and stored at 80°C until the biochemical parameters BUN, Cr, protein (Pro) and glucose were measured using a special laboratory kit.

Tests to measure oxidative stress markers in kidney tissue:

Measurement of lipid peroxidation in tissue: The level of lipid peroxidation is determined based on the formation of malondialdehyde. Malondialdehyde is a marker of oxidative stress and the final product of lipid peroxidation with thiobarbituric acid. For this purpose, after separation, the kidney (500 mg) was homogenized with ice cold 1.15% KCl solution (5 ml) and a homogeneous mixture containing 10% kidney tissue was obtained. Then, 0.5 ml of the 10% homogenate was placed in a tube and 3 ml of 1% phosphoric acid and 1 ml of 0.6% thiobarbituric acid were added to it. This mixture was heated at 100°C for 45 minutes. This reaction leads to the formation of malondialdehyde from lipid peroxides. Then, 4 ml of n-butanol was added to the cooled mixture and mixed well. After centrifugation, the absorbance of the n-butanol phase was read at 532 nm (24).

Measurement of reactive oxygen species production: 500 mg of kidney tissue was added to 5 ml of cold Tris-HCl buffer (40 mM, pH=7.4, 4°C) and homogenized with a homogenizer. 100 µl of the homogenate was added to 1 ml of cold Tris-HCl buffer (40 mM, pH=7.4) and 2′,7′-dichlorofluorescein diacetate (final concentration 1 µM). The samples were incubated for 15 min at 37°C in the dark. Finally, the fluorescence intensity of the samples was measured at an excitation wavelength of 485 nm and an emission wavelength of 525 nm using a fluorometer (25, 26).

Tests to measure the activity of antioxidant enzymes in kidney tissue: ELISA assay method was used to measure the activity of antioxidant enzymes. To lyse the tissue, the instructions of the kit manufacturer were used. First, we removed some kidney tissue and homogenized it with one milliliter of KCL solution and placed it in a microtube. In the next step, we centrifuged it for 4 minutes at 3000 rpm and then removed the upper solution and evaluated it. All data were statistically analyzed using one-way analysis of variance (ANOVA) and then Tukey's multiple comparison test, and $p<0.05$ was considered significant.

Results

Hematological and urinary parameters: Based on the results of tests performed on day 14 after surgery, plasma levels of BUN and Cr and urinary levels of Pro and glucose in the second group (BDL) whose bile

ducts were completely ligated were significantly increased compared to the Sham group ($p<0.001$). After administration of different doses of carvacrol, decreasing effects were observed compared to the second group (BDL) ($p<0.05$) (Figures 1 and 2).

Evaluation of oxidative stress indices: Evaluation of oxidative stress markers in all animals showed a significant increase in the production of reactive oxygen species and lipid peroxidation in cholestatic animals ($p<0.001$). It was observed that the use of carvacrol as a therapeutic strategy in animals with blocked bile ducts significantly reduced oxidative stress markers and related complications in cholestatic animals ($p<0.05$) (Figure 3).

Evaluation of antioxidant enzyme activity in kidney tissue: Based on the results of experiments performed on day 14 after surgery, the activity of glutathione peroxidase, superoxide dismutase, and catalase enzymes in the second group (BDL) whose bile ducts were completely ligated was significantly reduced compared to the Sham group ($p<0.001$). After intervention, cholestatic animals treated with carvacrol at different doses showed increased effects in all of the above parameters compared to the second group (BDL) ($p<0.001$) (Figure 4).

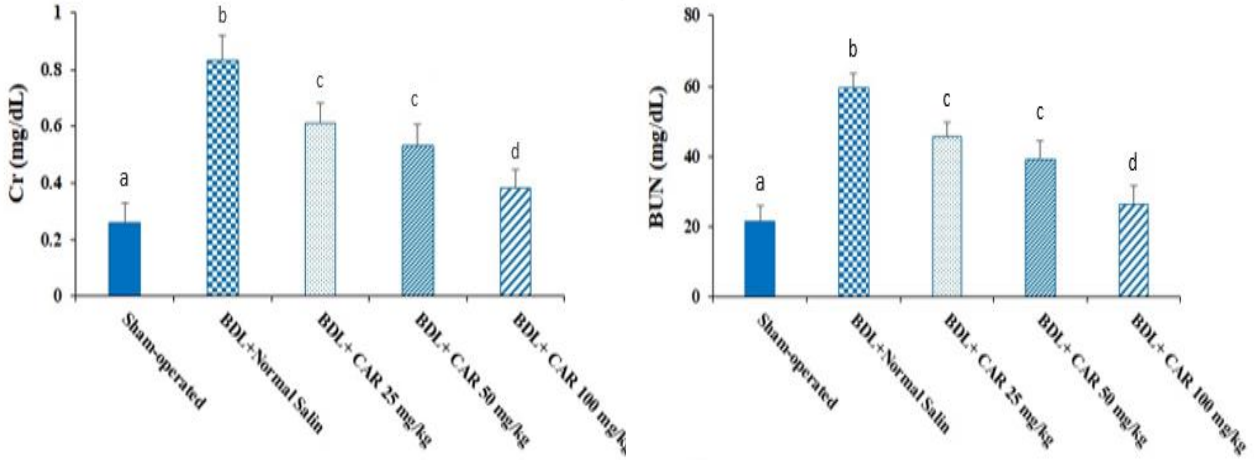

Figure 1. Serum biochemical changes in cholestatic animals (BDL) and the effect of carvacrol (CAR) administration. **Data are presented as Mean±SD for eight animals per group.**

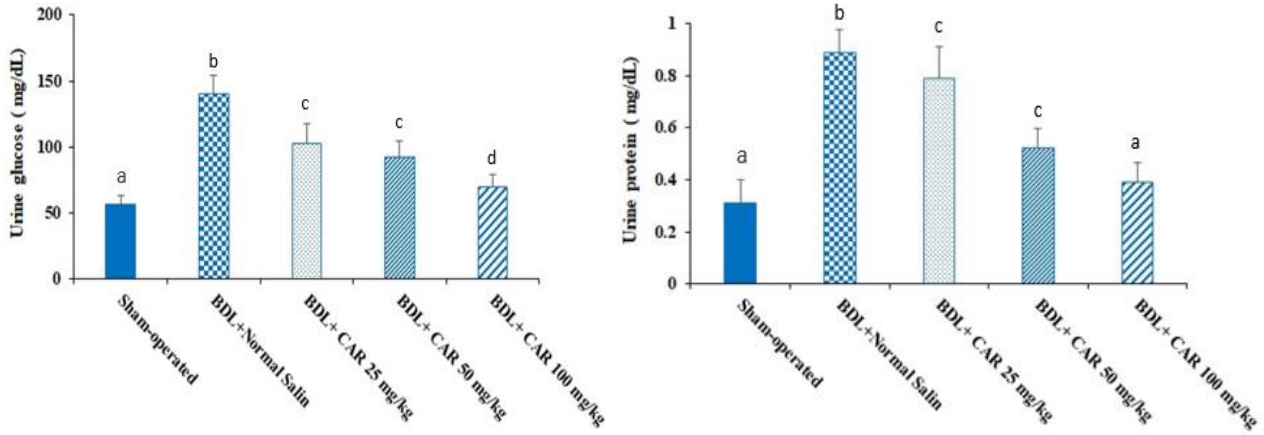

Figure 2. Changes in urinary protein and glucose levels in cholestatic animals (BDL) and the effect of carvacrol (CAR) administration on them. **Data are presented as Mean±SD for eight animals in each group.**

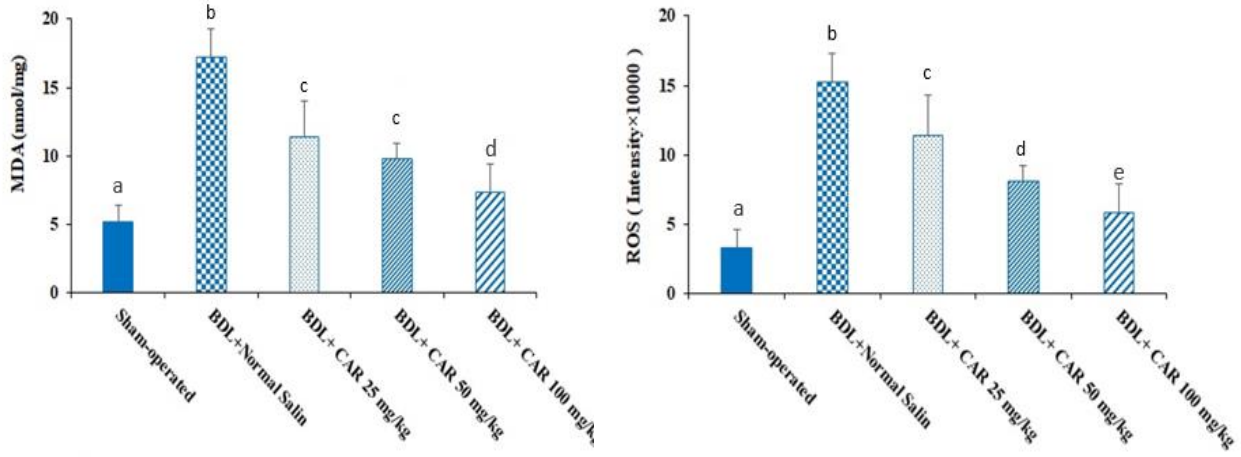

Figure 3. Evaluation of oxidative stress indices in kidney tissue in cholestatic animals (BDL) and the effect of carvacrol (CAR) administration on it. **Data are presented as Mean±SD for eight animals in each group.**

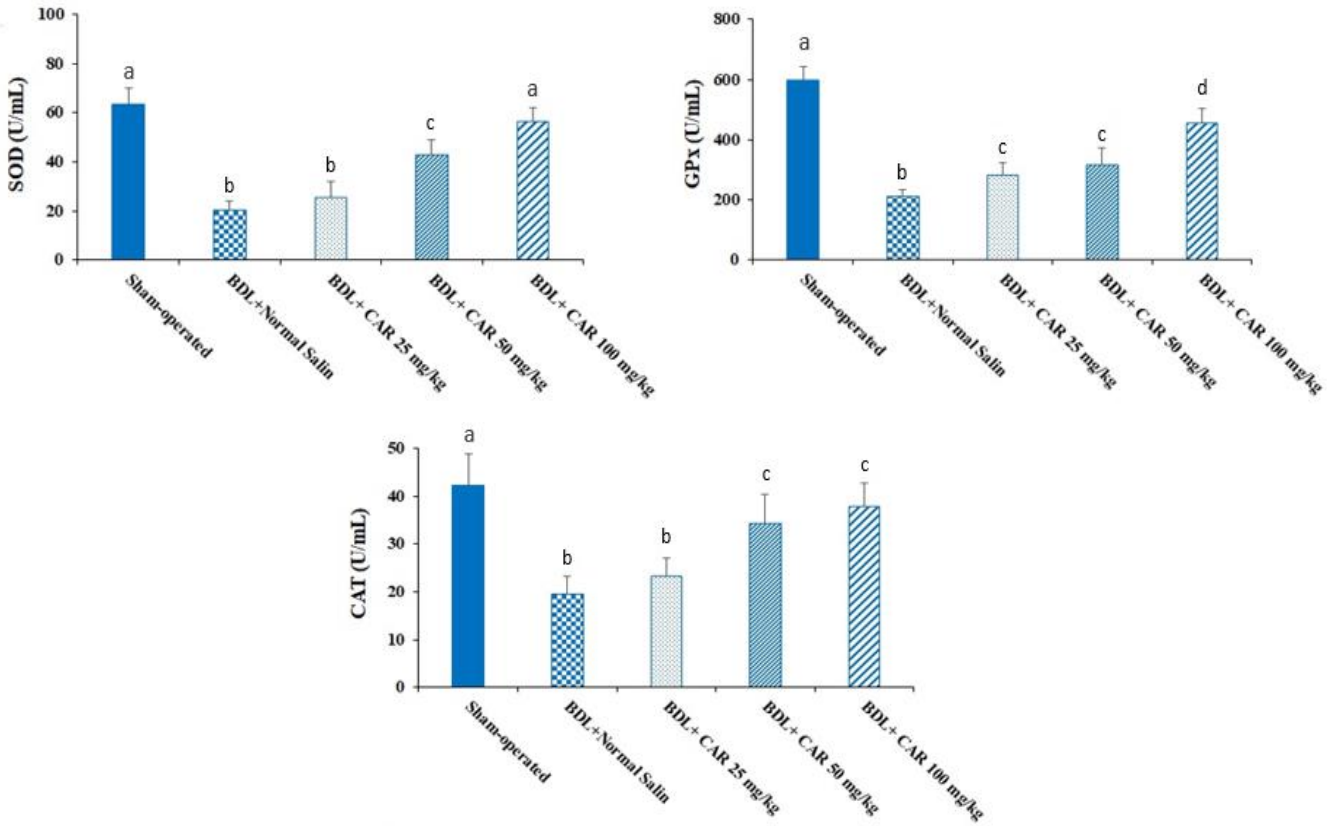

Figure 4. Evaluation of antioxidant enzyme activity in kidney tissue in different study groups and the effect of carvacrol on it. **Data are presented as Mean±SD for eight animals in each group.**

Discussion

In the present study, it was found that bile duct ligation in rats increased plasma BUN and Cr levels and increased glucose and Pro levels in urine. It was also observed that bile duct ligation increased the levels of oxidative stress markers and decreased the activities of antioxidant enzymes in rats undergoing renal injury. The results of the present study indicate an improvement in biochemical markers and oxidative stress following the consumption of different concentrations of carvacrol in rats undergoing renal injury caused by cholestasis. In a study conducted by Islam et al., it was found that bile duct ligation caused renal injury and increased BUN and Cr levels, which is consistent with our study (27). Furthermore, in the study of Ozturk et al., it was observed that bile duct ligation caused an increase in BUN and Cr and also increased glucose and Pro levels in urine in the studied animals (28), which was consistent with our study.

Bile duct ligation increases oxidative stress indices, which have been reported in several studies. This was also evident in our study. Oxidative stress, which is described as an uncontrolled increase in ROS production or a decrease in its elimination, plays a key role in the pathogenesis of acute kidney injury (29). Following kidney injury and increased levels of ROS, they attack biological macromolecules such as membrane lipids, nucleic acids, carbohydrates, and proteins (30). ROS also attack membrane lipids, resulting in the production of MDA, which is considered a predictor of structural oxidative damage in cell membranes (31).

The results of the present study indicate that the development of cholestasis increases oxidative stress in rat kidney tissue through increased ROS production and subsequent increased MDA production. The present study also showed that the activity of SOD, CAT, and GPx enzymes increases after acute kidney injury caused by cholestasis. The activity of SOD, CAT, and GPx enzymes plays an important role in preventing biological damage caused by increased ROS levels in the cell. Therefore, the SOD enzyme converts superoxide anions to H2O2, which is then converted to water by CAT and GPx (32). Moreover, the biochemical function of GPx is largely dependent on the level of intracellular glutathione (GSH) as the most important intracellular antioxidant molecule (33).

Carvacrol is a major natural component and is significantly present as an essential oil in aromatic plants and is known for its numerous biological activities (34). Studies have shown that the antioxidant activity of carvacrol is due to the presence of a free hydroxyl group and its hydrophobicity (35). It has also been shown that carvacrol reduces oxidative stress due to its strong antioxidant properties (36). The results of the present study showed that treatment of animals with different doses of carvacrol improved renal function in rats with cholestatic renal injury by reducing ROS levels and increasing the activities of antioxidant enzymes. Bakır et al. showed that carvacrol at different doses (50, 100, 200 mg/kg) reduced MDA levels and improved the activities of hepatic SOD, CAT and GPx enzymes in rats with Cerulein-induced acute pancreatitis. In addition to reducing the levels of hepatic aminotransferase enzymes, carvacrol also improved inflammation and necrosis in the liver (37).

In another study, Alvarenga et al. found that carvacrol improved intestinal inflammation induced by HCl in rats by reducing the production or release of proinflammatory cytokines interleukin-1 and TNF-α. Carvacrol also improved glutathione levels and reduced MDA and nitric oxide levels as markers of oxidative stress and inflammation (38). Ismail et al. also found that carvacrol could significantly increase the activity of three antioxidant enzymes, catalase, superoxide dismutase, and glutathione peroxidase, which are decreased in Candida auris fungal infections (39). In another study, de Santana Souza et al. concluded that carvacrol ameliorates acetic acid-induced oxidative damage by increasing the activity of antioxidant enzymes (catalase, superoxide dismutase) (40). The results of the study by Khalaf et al. showed that exposure to propiconazole leads to oxidative stress and lipid peroxidation, which was manifested by a

significant decrease in glutathione content and catalase activity and a significant increase in malondialdehyde content in the liver and kidney. These toxic effects were confirmed by histopathological studies. In contrast, carvacrol, due to its antioxidant properties, was able to reduce the harmful effects caused by propiconazole and improve tissue damage in the liver and kidney (41). This is consistent with the present study indicating a protective role of carvacrol by improving the activity of antioxidant enzymes and inhibiting the formation of free radicals in the damaged tissue.

Overall, the results of the present study showed that carvacrol treatment was able to significantly improve cholestasis-induced renal injury in rats. This protective effect may be due to the antioxidant effects of carvacrol. Consequently, carvacrol appears to be a very promising protective agent against cholestasis-induced renal injury. However, we suggest that further research be conducted to determine the feasibility and effectiveness of using carvacrol in clinical settings.

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