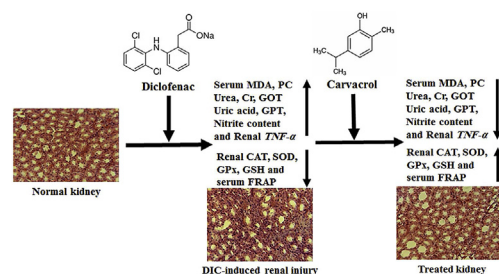


Research article

Carvacrol exerts nephroprotective effect in rat model of diclofenac-induced renal injury through regulation of oxidative stress and suppression of inflammatory response

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GRAPHICAL ABSTRACT



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ABSTRACT

Diclofenac (DIC) is an NSAID that can cause toxic effects in animals and humans and carvacrol (CAR) is a monoterpene compound that displays effective pharmacological and biological actions. The purpose of this work was to assess the influences of CAR on DIC-induced renal injury and oxidative stress in male rats. The rats were segregated into four groups. Group 1, control group; Group 2 received DIC-only; Groups 3, received CAR-only and group 4 received DIC plus CAR. Changes in biochemical indexes, pathological changes, molecular biological indexes, and genes related to the inflammation of main organs were evaluated. The results of this work indicated that the amounts of the serum protein carbonyl, sGOT, sGPT, urea, creatinine, uric acid, nitrite content, MDA, serum TNF- α , and renal TNF- α gene expression were remarkably increased and the levels of the GPx, GSH, CAT, and SOD were significantly reduced in DIC-only treated animals compared to the control group. On the other hand, treatment with CAR after exposure to DIC led to significant improvements in abnormalities of DIC-induced renal injury and serum biochemical factors. The data approve that CAR diminished the deleterious effects of DIC

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exposure. In this regard, the findings of this study indicated that the administration of CAR could alleviate the noxious effects of DIC on the antioxidant defense system and renal tissue.

1. Introduction

The kidneys are vital tissues in the body. Kidneys normalize acid-base balance, blood pressure, and body electrolytes. These tissues help the body as an intrinsic filter of blood and cleaner of medications or poisonous metabolites from the body [1]. They create some hormones and preserving the metabolism and creation of prostaglandins through cyclooxygenase (COX). The renal tissue also contributed to the hematopoietic system actions. All these fundamental actions of the renal tissue are essential for homeostasis. Renal damages often occur due to their contribution to detoxification, metabolism, and excretion of drugs and their metabolites, making the kidney an important target organ for drug-caused damages [1, 2, 3]. Renal injury is an important health disorder that is created through the use of some drugs, in particular nonsteroidal anti-inflammatory drugs (NSAIDs) [4, 5, 6].

NSAIDs are often prescribed for relieving symptoms of headaches, sprains and strains, painful periods, flu and colds, arthritis, and other causes of long-term pain [7] and they are among the most commonly used medications globally on a daily basis. NSAIDs are the most important toxicity-causing medications which in some cases can be fatal [8]. Prior researches have revealed that NSAIDs are among the most important medications that can provoke contrary drug reactions. Diclofenac (DIC), an NSAID, is universally consumed by many people mostly for the treatment of degenerative joint disease, pain, rheumatoid arthritis, trauma inflammation, and dysmenorrhea [9, 10]. Actually, worldwide DIC used annually has been estimated to be nearly 940 tons [11]. Although DIC is an effective remedial medication, its adverse effects in both humans and animals are related to prostaglandin biosynthesis suppression. Specifically, DIC-mediated hepatotoxicity, nephrotoxicity, and gastrointestinal injuries are the main side effects of the drug that are mostly induced due to the activation of oxidative damage [12, 13, 14]. Understanding that DIC causes its poisonous effects mostly through mechanisms induced by oxidative stress, clinicians, and researchers have emphasized the use of antioxidants as natural agents for the treatment of DIC-induced toxicity.

Carvacrol (CAR), 2-methyl-5-isopropylphenol, is a monoterpene compound found in essential oils of fragrant plants such as thyme, wild bergamot, oregano, and pepperwort with a distinguishing perfume of oregano [15]. CAR has been broadly used as a food additive for many years. Many studies have demonstrated that CAR displays effective pharmacological and biological actions such as; anticancer, antibacterial, anti-inflammatory, antifungal, antioxidant, spasmolytic, vasorelaxant, and hepatoprotective, both in vivo and in vitro [15]. Some researches revealed the ameliorative effects of this agent, including ischemia/reperfusion [16], gentamicin [17], and methotrexate-induced renal damage [18]. Due to the possible ameliorating effects of CAR on DIC-induced side effects, we aimed to investigate whether treatment of rats with CAR could prevent the cytotoxic effects of DIC on the kidney and suppress the incidence of oxidative stress.

2. Materials and methods

2.1. Animal care

To evaluate whether CAR could prevent DIC-induced kidney injury, thirty-two 6–8 weeks old Wistar rats with an estimated weight of around 200 ± 200 g were purchased from Pasteur Institute (Pasteur, Tehran, Iran). The standard condition was provided for all the rats, including $60 \pm 10\%$ humidity, 22 ± 2 °C environment temperature, and 12 h light/dark cycle. Rats also had the accessibility to the appropriate amount of food and water. Of note, animal care, and experimental procedures were

performed according to Guide for the Care and Use of Laboratory Animals published by the National Institute of Health (NIH) and Ethics Committee of Iran University of Medical Sciences, Tehran, Iran (Ethic number IR. IUMS. REC. 400. 123).

2.2. Experimental design

For conducting the investigation, we randomly categorized rats into 4 groups (each group contains 8 rats); control groups, which was included those rats that only received distilled water together with 5% DMSO solution (**Group 1**), rats that were treated with 10 mg/kg DIC dissolved in water (**Group 2**), rats which were orally treated with 10 mg/kg CAR dissolved in 5% DMSO solution (**Group 3**), and the rats that were simultaneously treated with both agents (**Group 4**). Of note, it should be noted that all the rats were subjected to the drugs (orally via gavage) for 14 consecutive days. In Group 4, rats were first exposed to DIC and an hour later, the desired dose of CAR was given to them. The dosage of both CAR and DIC were chosen according to the previous studies [19, 20, 21, 22]. After the drug treatment and keeping the animals in fast for 12 h, rats were killed, so that their renal tissues could be separated for further biochemical analysis. Their blood samples were also collected by the cardiac puncture method.

2.3. Investigating the kidney injury-associated serum markers

To investigate the effects of the drugs on kidney-associated serum markers, first, the serum was provided from the rat's blood specimen. Then, the serum levels of uric acid, Creatinine (Cr), glutamate oxaloacetate transaminase (GOT), and glutamate pyruvate transaminase (GPT) were measured using diagnostic kits (Pars Azmoon Co, Tehran, Iran) and an auto-analyzer system (BT3000, Roma, Italy).

2.4. Investigating serum antioxidant capacity

For evaluating the effects of drugs, either alone or in combination, on the serum antioxidant capacity, we used the ferric reducing/antioxidant power (FRAP) method according to the procedure that was explained previously [23]. FeSO₄ was used as a standard of FRAP assay.

2.5. Biochemical analyses

A slice of renal specimens was weighed and homogenized in ice-cold phosphate buffer (pH 7.2). The homogenates were centrifuged at 4500 rpm/10 min applying a cooling centrifuge at 4 °C, and then the supernatants were applied to assess the biochemical markers (MDA, GSH, CAT, SOD, GPx, and nitrite). All tests were performed in triplicate.

Kidney and serum MDA were evaluated by determining the formation of thiobarbituric acid (TBA) (Merck, Darmstadt, Germany) reactive substances [24]. To do so, we mixed 100 μ L of the kidney homogenate or serum with the equivalent amount of sodium dodecyl sulfate (SDS) (Merck, Darmstadt, Germany) and 2.5 mL of TBA. The mixture was then heated at 95 °C for 1 h and then was centrifuged at 4000 rpm for 10 min. The optical density (OD) was then measured at 532 nm.

The content of glutathione (GSH) in renal tissue was quantified according to the Ellman protocol [25] based on the use of 5,5-dithiobis-2-nitrobenzoic acid (DTNB or Ellman reagent). Briefly, 100 μ L of supernatant was diluted with 4.0 mL of Tris buffer (0.4 M, pH: 8.9), and 0.1 mL of DTNB. GSH level was assessed by a spectrophotometer at 412 nm, and the renal content of GSH was reported as μ mol/g wet tissue.

The method developed by Flohé was used to evaluate the enzymatic activity of SOD in renal tissue [26]. First, the protein was extracted from the tissue samples and the amount of extracted proteins was evaluated

Table 1. Nucleotide sequences of primers used for real-time RT-PCR.

| Gene | Forward primer (5'-3') | Reverse primer (5'-3') |
|----------------|------------------------|------------------------|
| β -actin | AGGAGTACGATGAGTCCGGC | CGCAGCTCAGTAACAGTCCG |
| TNF- α | CTGGCGTGTTTCATCCGTTTC | GGCTCTGAGGAGTAGACGATAA |

according to the Bradford protocol [27]. The inhibition of nitro blue tetrazolium (NBT) reduction (Sigma-Aldrich Company, Burlington, USA) by SOD in each sample was quantified spectrophotometrically at 560 nm.

The CAT activity was assessed using the method of Aebi [28]. To evaluate the reduction of H₂O₂ in the tissue, we added 995 μ L H₂O₂ solution (composed of: 10 mmol H₂O₂ in 50 mM phosphate buffer, pH 7.4) to 5 μ L homogenate and then the optical density (OD) was measured at 240 nm.

To evaluate the activity of GPx, we incubated the samples with NaNO₃ and H₂O₂ as described previously [24]. At each time interval, 0.1 mL of homogenate was incubated with 0.2 mL of ethylene diamine tetraacetate, sodium azide, and H₂O₂ together with 0.4 mL of phosphate buffer. After adding TCA, as a stopping reagent, the mixture was centrifuged at 2000 rpm. Supernatant was collected and mixed with disodium hydrogen phosphate and DTNB. Once the color was formed, the absorbance was measured at 412 nm. The data are presented as U/mg protein.

The serum level of protein carbonyl (PC) was measured according to the method described by Reznick and Packer [29]. The collected serums from the animals were divided into two tubes. While in the first tube, serum was mixed with 4 mL of 2,4-dinitrophenylhydrazine (DNPH)/L in HCl/L, in the second tube, it was incubated with HCl/L. After 1 h of incubation in the dark at room temperature and being vortexed every 15 min, each tube was re-incubated with 5 mL trichloroacetic acid solution (20%) for 10 min on ice. Tubes were centrifuged at 3000 \times g for 5 min and then, were washed with 10% trichloroacetic acid. After mechanically breaking the protein pellets and washing for 3 times with ethanol-ethyl acetate, the pellets were dissolved in guanidine hydrochloride. The absorbance was measured at 360 nm. The data are presented as nmol DNPH/mg protein.

The amount of Nitrite was quantified by determining the optical density at 548 nm after incubating the samples with Greiss (Sigma-Aldrich Company, Burlington, USA) reagent as described previously [24]. 200 μ L of Griess reagent was added to the supernatant and the mixture was incubated for 30 min at 37 °C. OD was read at 548 nm. Using a standard curve of sodium nitrite, we normalized the amount of nitrite content. The reported amount of nitrite is presented as μ M/mg tissue.

2.6. Evaluating the amount of TNF- α in serum and renal tissue

To test the amount of TNF- α in the serum and renal tissue of drug-treated rats, we used an ELISA-based TNF- α kit (BT-Laboratory, Shanghai, China).

2.7. Analyzing gene expression using real-time PCR

After extracting RNAs from the renal tissue using RNX-Plus Solution (Sina Clon, Tehran, Iran), and evaluating the quality of RNAs using

Nanodrop2000 (ThermoFisher, Waltham, USA), the relevant cDNAs were synthesized using PrimeScript™ reagent kit (Takara, Nijigahashi, Japan). The prepared cDNAs were then amplified by SYBR® Green PCR Master Mix (Qiagen Co., Hilden, Germany) in the presence of the primers, which were designed by Oligo 7.0 software and NCBI BLAST. Table 1 summarized the sequence of used primers in the present study. The thermal cycling condition of qRT-PCR analysis was as follows; denaturation at 95 °C for 15 s, annealing at 60 °C for 20 s, and extension at 72 °C for 25 s. The alteration in gene expression was measured according to the 2^{- $\Delta\Delta$ ct} formula [30].

2.8. Histopathological study

For histological examines, sections of renal tissue were fixed in 10% formalin for one week at 4 °C. Tissues were removed from formalin after 1 week and dehydration-rehydration processes were performed to fix the samples. After the tissues were placed in a paraffin block (Merck, Darmstadt, Germany), tissue sections were prepared using a microtome device (AMR 400, Amos Scientific, Melbourne, Australia) and then the slides were stained with hematoxylin-eosin. Each slide was then examined by a pathologist using a light microscope (Nikon Eclipse E400, Wilmington, USA) at different magnifications [3].

2.9. Statistical analysis

All the experiments in the present study were performed in triplicate to obtain statistically meaningful results. Data are expressed as Mean \pm SD according to one-way ANOVA and Tukey's post hoc tests. SPSS 18 software was also used for data analysis. A probability level of P < 0.05 was considered statistically significant.

3. Results

3.1. Effect of CAR on serum levels of Cr, GPT, urea, uric acid, and GOT

We found that DIC treatment for 14 days increased the serum levels of urea, GOT, GPT, Cr, and uric acid in rats (P < 0.05) as compared to the control group (Table 2); suggestive of the probable adverse effect of the drug in kidney function. However, it seems that CAR could remarkably prevent DIC-induced renal toxicity, as the serum levels of aforesaid parameters was significantly diminished, when rats were exposed to CAR post-DIC treatment.

3.2. The protective effect of CAR on plasma antioxidant capacity, nitrite content, and MDA levels

As presented in Table 3, our data showed that the serum antioxidant capacity was significantly decreased in rats, which were treated with DIC for 14 days, as compared to the control group (P < 0.05). In agreement with this finding, the significant increase in tissue levels of nitrite, MDA, and serum MDA also suggested that DIC could remarkably induce kidney injury, at least partly, through inducing oxidative stress. On the other

Table 2. Effect of carvacrol on some serum biochemical parameters in the experimental groups.

| Parameters | Group 1 | Group 2 | Group 3 | Group 4 |
|-------------------|-----------------|---------------------------------|--------------------------------|---------------------------------|
| Urea (mg/dl) | 30.6 \pm 1.9 | 51.7 \pm 4.5 ^{###} | 29.9 \pm 2.7 ^{***} | 32 \pm 3.6 ^{***} |
| Cr (mg/dl) | 0.79 \pm 0.16 | 1.82 \pm 0.21 ^{###} | 0.82 \pm 0.14 ^{***} | 0.85 \pm 0.19 ^{***} |
| Uric acid (mg/dl) | 1.26 \pm 0.09 | 2.41 \pm 0.25 ^{###} | 1.12 \pm 0.16 ^{***} | 1.37 \pm 0.14 ^{***} |
| sGPT (U/L) | 71.5 \pm 8.7 | 128.5 \pm 4.3 ^{###} | 70.9 \pm 9.5 ^{***} | 71.9 \pm 8.8 ^{***} |
| sGOT (U/L) | 134.1 \pm 7.8 | 281.2 \pm 24.1 ^{###} | 136.9 \pm 8.7 ^{***} | 148.7 \pm 15.8 ^{***} |

Data are expressed as mean \pm SD (n = 8) and analyzed by one-way ANOVA followed by Tukey post hoc test. Group 1: control; group 2: diclofenac only; group 3: carvacrol only and group 4 were treated by diclofenac plus carvacrol.

^{###} p < 0.001 versus control group (Group 1).

^{***} p < 0.001 versus diclofenac-only administered group (Group 2).

Table 3. Effect of carvacrol on ferric reducing/antioxidant power (FRAP), Nitrite content and malondialdehyde (MDA) levels in the experimental groups.

| Parameters | Group 1 | Group 2 | Group 3 | Group 4 |
|--|------------------|---------------------------------|----------------------------------|--------------------------------|
| Serum FRAP ($\mu\text{mol/L}$) | 609.1 \pm 99.2 | 389.2 \pm 40.6 ^{###} | 644.2 \pm 122.5 ^{***} | 601 \pm 67.5 ^{***} |
| Nitrite content ($\mu\text{M/mg}$ tissue) | 6.87 \pm 0.36 | 13.69 \pm 0.43 ^{###} | 6.74 \pm 0.46 ^{***} | 6.91 \pm 0.32 ^{***} |
| Serum MDA (nmol/L) | 1.03 \pm 0.16 | 5.95 \pm 0.91 ^{###} | 1.05 \pm 0.19 ^{***} | 1.28 \pm 0.27 ^{***} |
| Kidney MDA (nmol/mg protein) | 1.56 \pm 0.24 | 3.90 \pm 0.33 ^{###} | 1.39 \pm 0.46 ^{***} | 1.66 \pm 0.25 ^{***} |
| Protein carbonyl (nmol NADPH/mg protein) | 4.94 \pm 0.86 | 12.22 \pm 0.85 ^{###} | 5.06 \pm 0.91 ^{***} | 5.86 \pm 0.92 ^{***} |

Data are expressed as mean \pm SD (n = 8) and analyzed by one-way ANOVA followed by Tukey post hoc test. Group 1: control; group 2: diclofenac only; group 3: carvacrol only and group 4 were treated by diclofenac plus carvacrol.

^{###} p < 0.001 versus control group (Group 1).

^{***} p < 0.001 versus diclofenac-only administered group (Group 2).

hand, we found that when rats were exposed to CAR after DIC, not only there was a significant elevation in serum antioxidant capacity (P < 0.05) but also the tissue levels of nitrite content, MDA, and serum MDA robustly declined as compared to the DIC-alone treated group (P < 0.05) (Table 3).

3.3. Effect of CAR on serum protein carbonyl

In agreement with the previous findings, our results showed that while DIC (100 mg/kg) could significantly increase the serum PC content in rats (Table 3) (P < 0.05) as compared to the control group, the companionship of CAR with this agent could remarkably decrease the serum level of this protein in the rats (P < 0.05). It should be noted that we failed to find any obvious difference between the serum level of PC in CAR-treated rats and the control group.

3.4. Effect of CAR on CAT, SOD, GPx activities, and GSH level

Rats that were treated with a single agent of DIC showed a significant decrease in renal CAT and SOD activities, as compared to the control group (P < 0.05) (Table 4). However, the results obtained in the DIC-plus-CAR group were completely different, as CAR could remarkably elevate kidney SOD and CAT activities in rats, as compared to the DIC-alone group (P < 0.05) (Table 4). To confirm the results, we also evaluated the effects of CAR and DIC, either alone or in combination, on kidney GPx activity. We found that while DIC reduced the intracellular amount of GSH in the kidney tissue (P < 0.05), the administration of CAR at the concentration of 10 mg/kg after DIC exposure could remarkably ameliorate the anti-oxidant condition of renal tissue through elevating the level of GSH (p < 0.05) (Table 4).

3.5. Effect of CAR on the expression of TNF- α in rats

Having established the effects of CAR and DIC on the anti-oxidant activity of renal tissue in rats, it was of particular interest to evaluate the impacts of both drugs on the serum and tissue level as well as the expression of TNF- α . DIC not only remarkably increased the expression

level of TNF- α (Figure 1A) but also led to an increase in the serum and tissue level of this inflammatory cytokine in rats (Figure 1B and C) (P < 0.01). Interestingly, when we treated rats with CAR after DIC exposure we found that both the expression level and the tissue level of TNF- α significantly reduced as compared to DIC-treated rats (Figure 1A and C) (P < 0.01). It should be noted that CAR was able to diminish the tissue level of TNF- α in rats after DIC exposure to the same level that was observed in the control group.

3.6. Histopathological findings

We also evaluated whether DIC and CAR, either as a single agent or in combination, could change the morphology of kidneys in rats. As presented in Figure 2A, while both the control group and CAR-treated groups showed to have a normal morphology of the kidney, the kidney morphology of DIC-treated rats showed the infiltration of lymphocyte cells in the tissue (Figure 2A, B, and C). Of note, the administration of CAR to rats after DIC exposure significantly eliminated the population of lymphocytes and degenerated cells in the kidney tissue (Figure 2D); indicative of the ameliorative impacts of CAR on devastating effects of DIC in the rats.

4. Discussion

The current research discloses novel evidence regarding the protective effects of CAR on DIC-induced nephrotoxicity through regulating oxidative stress as well as inflammatory responses.

Cr, urea, and uric acid contents are applied as serum biochemical markers of kidney injuries; accordingly elevated levels of these factors may reveal kidney deficiencies [31]. Creatinine is a non-protein nitrogenous agent that is composed of phosphocreatine and creatine in the process of muscle metabolism and some of it is secreted through glomerular filtration [32]. In similarity to urea, it seems that the Cr secretion could also be influenced by glomerular filtration rate (GFR), suggesting that any modification that diminishes GFR leads to an increase in serum creatinine [33]. The considerable elevation in the amount of creatinine, urea, and uric acid after exposure of rats to DIC

Table 4. Effect of carvacrol on catalase (CAT) activity, superoxide dismutase (SOD) activity, glutathione peroxidase (GPx) activity and Intracellular glutathione (GSH) level in the experimental groups.

| Parameters | Group 1 | Group 2 | Group 3 | Group 4 |
|---------------------------------|------------------|-------------------------------|---------------------------------|---------------------------------|
| CAT (U/mg protein) | 175.8 \pm 11.6 | 52.9 \pm 7.3 ^{###} | 177.9 \pm 14.3 ^{***} | 177.8 \pm 10.3 ^{***} |
| SOD (U/mg protein) | 32.9 \pm 3 | 14.5 \pm 1.1 ^{###} | 33.5 \pm 3.3 ^{***} | 34.5 \pm 2.2 ^{***} |
| GPx (U/mg protein) | 24.9 \pm 1.5 | 14.9 \pm 0.9 ^{###} | 25.1 \pm 1.4 ^{***} | 26.1 \pm 1.2 ^{***} |
| GSH ($\mu\text{mol/g}$ tissue) | 12.9 \pm 1.5 | 5.9 \pm 0.3 ^{###} | 12.9 \pm 0.5 ^{***} | 14.5 \pm 1.3 ^{***} |

Data are expressed as mean \pm SD (n = 8) and analyzed by one-way ANOVA followed by Tukey post hoc test. Group 1: control; group 2: diclofenac-only; group 3: carvacrol-only and group 4 were treated by diclofenac plus carvacrol.

^{###} p < 0.001 versus control group (Group 1).

^{***} p < 0.001 versus diclofenac-only administered group (Group 2).

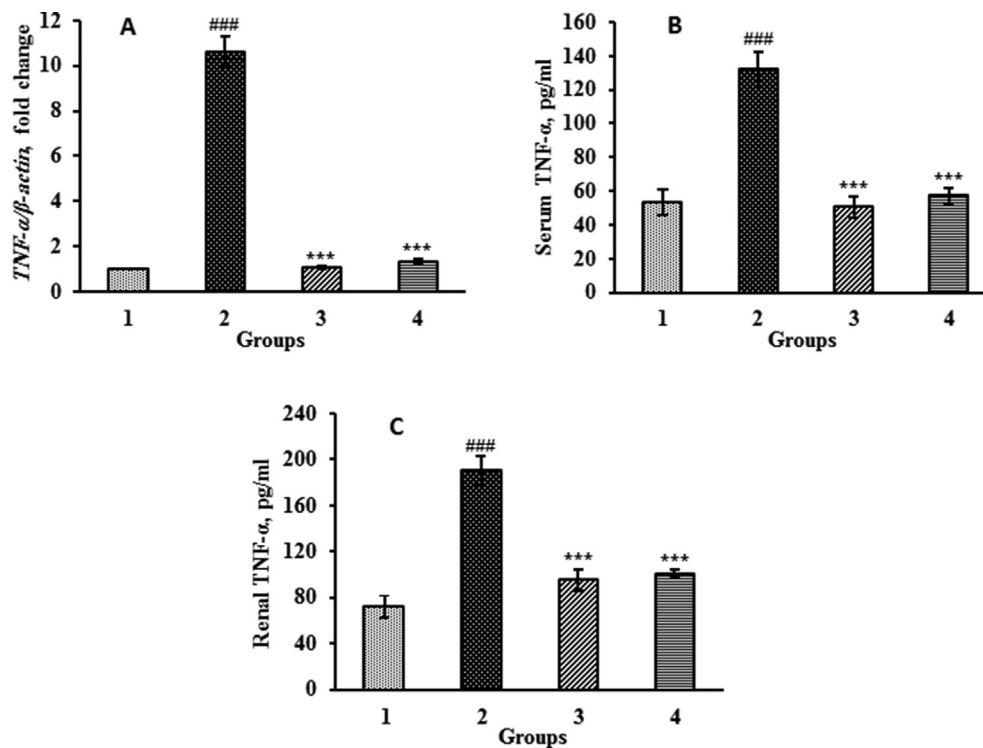


Figure 1. Effect of carvacrol on gene expression of TNF- α (A), serum TNF- α (B) and renal TNF- α (C). Each value represents the mean \pm SD of eight rats. Group 1: control; group 2: diclofenac-only; group 3: carvacrol-only and group 4 were treated by diclofenac plus carvacrol. ^{###} $p < 0.001$ versus control group (Group 1). ^{***} $p < 0.001$ versus diclofenac-only administered group (Group 2).

may be a sign of kidney impairment. These findings suggested that probably DIC leads to a reduction in GFR of the animals, which in turn caused the elevation in the serum levels of some biochemical marker [34, 35, 36]. On the other hand, when CAR could diminish the serum levels of Cr, urea, and uric acid in rats after exposure to DIC, it could be assumed that probably this agent might have an ameliorative effect on DIC-induced nephrotoxicity through increasing GFR.

We also reported a considerable augmentation in GPT and GOT contents, two important biochemical markers indicating hepatocellular and renal damages [34], in those rats which were exposed to the

single-agent of DIC. This finding was in agreement with the results reported previously [37, 38]. Of note, our results suggested that CAR could effectively reduce DIC-mediated elevation of GPT and GOT in rats. Given the results obtained from the previous studies suggested the protective impacts of anti-oxidant compounds on the serum level of these two enzymes [39, 40], it is reasonable to assume that probably the ameliorating impact of CAR on DIC-induced renal injury is mediated through regulating anti-oxidant responses within the kidney tissue.

The amount of Malondialdehyde (MDA) is an indicator of lipid peroxidation (LPO), which shows the activation of oxidative stress within

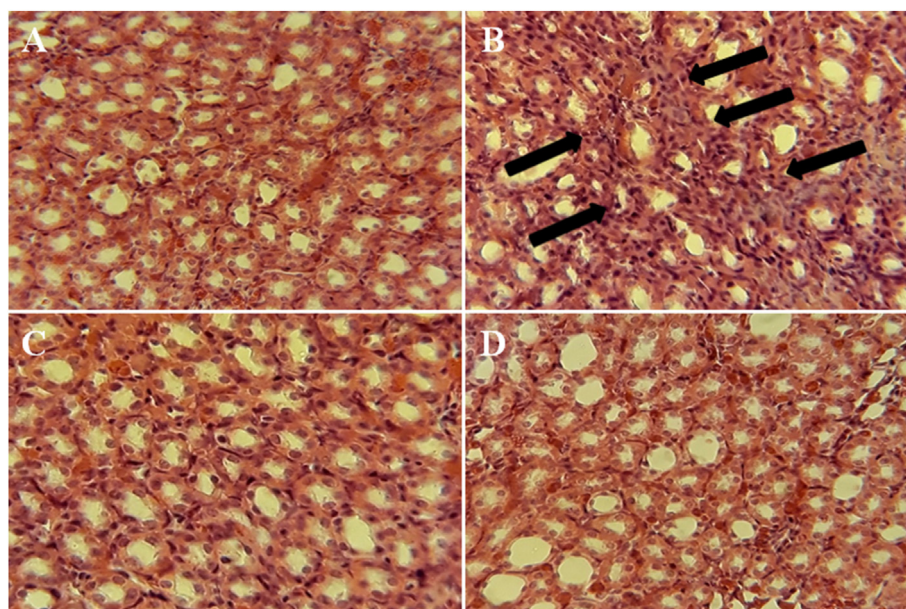


Figure 2. Effects of carvacrol on the renal histology of experimental groups. (A) Control group with normal structure; (B) diclofenac-only administered rats show lymphocyte infiltration (the black arrows); (C) carvacrol-only administered rats; (D) diclofenac-administered rats supplemented with carvacrol.

the cells [41]. In this study, the amount of MDA was considerably augmented in the DIC-treated rats, which was in accordance with the results of the earlier researches [34, 38]. Furthermore, treatment with CAR after exposure to DIC not only caused a rise in FRAP level but also caused a decline in MDA content in the renal tissues and sera. The protective impact of CAR on the kidney tissue could be attributed to the ability of the agent in neutralizing the free radicals. The same results were obtained when we evaluated the effects of both CAR and DIC on the nitrite level of rats and we found that DIC could elevate the level of nitrite in rats. This finding was in total agreement with the previous results disclosed that NO plays a vital role in DIC-induced damage [42, 43]. Moreover, we found that CAR could remarkably diminish the levels of NO in DIC-treated rats, shedding another light on the anti-oxidant property of the agent.

The enzymes of SOD and CAT are substantial factors in the system of antioxidant defense. Two superoxide radicals ($O_2^{\bullet-}$) combined with SOD and produce Hydrogen peroxide (H_2O_2). Finally, hydrogen peroxide in peroxisomes is converted by CAT to H_2O and oxygen molecules [44]. Several surveys have disclosed that DIC diminishes the activity of antioxidant enzymes in the renal tissue [37, 45]. On the other hand, there is a compelling number of studies indicating that CAR has the ability to reinforce the activity of enzymes that are responsible for regulating anti-oxidant responses [19, 22]. The results of the present study also showed that while DIC decreased the activity of SOC and CAT in rats and thereby produced oxidative stress, this was CAR that could evolve an anti-oxidative defense against DIC-induced nephrotoxicity through elevating the activity of SOD and CAT.

Additionally, the inordinate creation of free radicals as a result of DIC administration was associated with the elevated protein-oxidation reaction, which in turn lead to the augmentation of PC contents, indicating that protein oxidation could be one of the mechanisms that participated in DIC-induced renal injury. These observations are in accordance with earlier researches [34, 46]. In this study, the administration of CAR decreased DIC-mediated oxidative stress in the renal tissue by reducing the amount of PC. It should be noted that previous studies declared that CAR serves as a ROS scavenger and is capable to stabilize membrane structures.

Glutathione (GSH), an endogenous antioxidant, plays a chief function in protecting cells from oxidative stress-induced tissue injury. Thus far, several models have determined that DIC could induce oxidative stress by altering the intracellular level of GSH within the cells [47, 48]. In agreement, we also found that DIC-induced kidney damage resulted in a considerable augmentation in the amount of GSH in renal tissues. In contrast, a considerable restoration of GSH content was discovered in CAR administrated animals. The restoration of GSH content caused by CAR could be due to either the ability of the drug in augmentation of GSH level or due to the protective effect of the drug on oxidative stress.

To investigate the role of CAR on GSH metabolism, we tested the effect of the drug on GPx, an enzyme that deactivates peroxides by converting GSH to oxidized glutathione (GSSG). DIC-caused differences in the activity of GPx have been explained formerly in renal and hepatic tissues [37, 47]. In the current research, DIC-caused diminution of GPx activity probably to induce oxidative stress and on the other hand, CAR increased the activity of this enzyme to provide a defense against DIC-induced oxidative stress.

Several surveys have also revealed that DIC motivates the movement of monocytes and macrophages, which results in the production of diverse pro-inflammatory cytokines, including $IL-1\beta$, $NF-\kappa\beta$, and $TNF-\alpha$ [37, 49]. Some reports also disclosed that $TNF-\alpha$ plays an important role in the formation of inflammatory responses, especially in kidney tissue [34, 46]. The data of the present investigation confirmed that exposure to DIC considerably elevated the $TNF-\alpha$ gene expression in the kidney tissue. Conversely, administration of CAR after exposure to DIC considerably diminished $TNF-\alpha$ gene expression. We also reported that CAR could ameliorate DIC-induced kidney damage by hampering the inflammatory response. When tissue injury takes place, leucocytes rapidly migrate into

the injury site and trigger inflammatory responses [50, 51]. As previously shown in a histological study, leucocyte infiltration was considerably raised in the renal tissue of DIC-administrated animals. Nevertheless, in this research administration of CAR noticeably diminished leucocyte infiltration in the kidney of the animals receiving DIC, suggestive of the anti-inflammatory property of CAR.

5. Conclusion

Our data revealed that administration of CAR to rats that were previously exposed to DIC could remarkably ameliorate the devastating impact of DIC on kidney function, as this agent significantly increased the levels of CAT, GSH, GPx, and SOD, while it reduced the serum levels of Cr, PC, GPT, uric acid, urea, MDA, and GOT. Apart from the restoration of anti-oxidant compounds within the serum of rats, CAR also showed to have the ability to reduce DIC-induced elevated levels of $TNF-\alpha$. This finding adds another dimension to the nephroprotective property of CAR, this time from the perspective of an anti-inflammatory agent. Overall, our findings suggested that CAR could be a good candidate to be administrated alongside DIC to prevent its unfavorable nephrotoxicity side effects.

Declarations

Author contribution statement

Ali Nouri: Performed the experiments; Wrote the paper.
 Farzad Izak-Shirian: Performed the experiments; Wrote the paper.
 Vahideh Fanaei, Alireza Moradi: Conceived and designed the experiments.
 Maryam Dastan: Contributed reagents, materials, analysis tools or data.
 Mahdieh Abolfathi: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.
 Mansoor Khaledi, Hamzeh Mirshekari-Jahangiri: Conceived and designed the experiments; Analyzed and interpreted the data.

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