Research Article

Renoprotective Effects of Origanum majorana Methanolic L and Carvacrol on Ischemia/Reperfusion-Induced Kidney Injury in Male Rats

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1.Introduction

Ischemic acute renal failure (ARF) occurs after a sudden drop in blood flow to the whole or part of the kidney [1]. ARF is a severe decrease in renal function that happens due to significant necrosis in renal tubules [2]. In developed countries, the causes of death in ARF patients include respiratory failure, dialysis, and sepsis [3]. The most important cause of ARF in normal kidneys and in transplanted kidneys is kidney ischemia-reperfusion (I/R) damage. There is no specific treatment for ARF patients [4]. I/R injury occur in patients with severe stress such as kidney transplantation, acute rejection in kidney transplantation, cardiovascular surgery, trauma, and renal failure [5, 6]. With the closure of renal blood flow in ischemic ARF, chain events occur that eventually lead to kidney damage [7]. Components have a vital role in I/R include reactive oxygen species (ROS), activated neutrophils, and purine metabolites [2, 7]. When arterial blood flow is reestablished, oxygen reaches the kidney tissue through the bloodstream and forms free radicals. Free radicals increase, which is much more than the capacity of cellular detoxification in the kidneys, resulting in cellular damage [8].

For partial reversal of renal function due to I/R, removal of free radicals is used as the first method in preventing tissue damage after organ transplantation [7]. Antioxidants play a vital role in removing free radicals or cutting off the chain oxidation reactions inside and outside the body [9]. An imbalance between the antioxidant system and ROS is well-known as oxidative stress and can lead to oxidative injury. The ROS can disrupt the function of lipids, DNA, and proteins and depletes antioxidant enzymes [10].

In several studies, the protective effect of saffron (Crocus sativus L.) [11], Malva sylvestris L. [12], Salvia miltiorrhiza

Reference

[1]...
[13], and Nigella sativa L [14] have been investigated on the I/R model. Previous studies indicated that ROSs play a major role in the pathogenesis of I/R. Also, the protective impacts of antioxidants on I/R damage in the heart, liver, intestines, and kidneys have been proven, and the treatment with antioxidants minimizes I/R damage [2, 8]. *Origanum majorana* L (OM) is one of 200 genera in the family Lamiaceae of 3500 species spread over the world [15, 16]. It is a perennial plant native to the Mediterranean and southern Europe. Traditionally, it is utilized as a folk remedy against rheumatism, asthma, chest infection, headache, cough, sore throat, nervous disorders, epilepsy, and stomach disorders [15]. It has antioxidant activity and free radicals scavenging ability [15]. Carvacrol (CAR) is normally found in different plants of Lamiaceae family. It has anti-inflammatory, antioxidant, antitumor, cytoprotective, and antimicrobial activities [17]. α-Tocopherol (Vit E) is located in the cell membranes and protects the membrane lipids against oxidative injury [18]. Because medical treatment and common chemical drugs have many side effects and complications, as well as high financial burdens, the tendency toward traditional treatment and use of medicinal plants is increasing in the world. There is no information on the impact of OM in the renal I/R injury. We aimed to evaluate the impacts of OM leaves methanolic extract, CAR and Vit E on renal injuries induced by I/R in male Wistar rats.

2. Materials and Methods

2.1. Plant Material. The leaves of *Origanum majorana* were collected in spring 2019, from Yazd mountain (Yazd, Iran) and it was identified by the botanist (Herbarium no; MPISB-158).

2.2. Plant Extraction. The leaves of plant (200 g) were shade dried, powdered and soaked in 80% (v/v) methanolic solution at 25°C for 72 h under shaking condition. The supernatant was concentrated in a rotary evaporator and dried in 37°C. The dry extract was stored at −20°C for this study [19].

2.3. Chemicals and Reagents. 2, 4-dinitrophenylhydrazine (DNPH), Trichloroacetic acid (TCA), and formaldehyde were acquired from Merck (Germany). Carvacrol (98%), α-tocopherol (96%), pentobarbital, (5, 5′-dithiols-(2-nitrobenzoic acid)) (DTNB), thiobarbituric acid (TBA), and Ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma Chemical Co (St. Louis, MO, USA). All other chemicals and reagents utilized were of analytical mark.

2.4. Animals. Adult male Wistar rats (weighing 300 ± 50 g) were purchased from the Isfahan University of Medical Sciences (Isfahan, Iran) Animal House and were adjusted for 10 days with permitted access to water and standard diet at 12 h light and dark cycle. All procedures were accepted by the Ethics Committee of Yasuj University of Medical Sciences (Ethical code; IR.YUMS.REC.1398.138).

2.5. Experimental Procedure. Thirty male Wistar rats were randomly allocated into five groups (6 rats in each group) as follows:

(i) Group I (sham control): received olive oil intraperitoneally 72, 48, and 24 h before the study and immediately after surgery

(ii) Group II (I/R, positive control): received olive oil intraperitoneally 72, 48, and 24 h before the study and immediately after I/R

(iii) Group III (300 mg/kg extract): received OM methanolic extract (300 mg/kg Body weight, BW) [20] orally 72, 48, and 24 h before the study and immediately after I/R

(iv) Group IV (75 mg/kg CAR): received CAR (75 mg/kg BW) [17] intraperitoneally 72, 48, and 24 h before the study and immediately after I/R

(v) Group V (Vit E 100 mg/kg): received Vit E (100 mg/kg BW) [21] intraperitoneally 72, 48, and 24 h before the study and immediately after I/R.

The rats were anaesthetized by pentobarbital sodium (60 mg/kg) after prep and drep under sterile condition, the abdomen was opened, and the right kidney was removed (all groups); then the artery of the left kidney was ligated for 30 minutes (except for the sham group).

The animal was permitted to recover from anaesthesia prior to returning to its dedicated cage. 24 h after reperfusion, the rats were reanaesthetized by pentobarbital sodium, tracheotomized; then, a mask connected to the oxygen tank was placed on a tracheal tube for oxygenation. The rectal probe was put into the rectum to keep its temperature at 37°C. A cannula was set in the right femoral vein to infuse normal saline (3 ml/h) and a bolus of pentobarbital as necessary by a syringe-pump. A right femoral artery was also cannulated and linked to a pressure transducer (MLT844; AD instrument, Australia) for continuous recording of blood pressure (PowerLab/USP data acquisition system, AD instrument Australia). Afterward, the bladder was cannulated and the animals were allowed to have 1 h of equilibration. Then, a clearance period with 2 h duration was taken to collect urine in a preweighed container. At the finale of the clearance period, a blood sample (5 ml) was taken from the arterial cannula, centrifuged, and the plasma was kept at −20°C until assayed. The collected urine was weighed and diluted and kept in the refrigerator. Finally, the left kidneys were removed, weighed, and cut out into two sections; one part frozen in liquid nitrogen for redox indices and the other one kept in formalin (10%) for pathologic studies.

2.6. Biochemical Analysis. Urine and plasma samples were examined for creatinine (Cr) and urea using commercially available kits (Pars Azmoon, Iran). Potassium (K) and sodium (Na) were determined with the ion-selective electrodes technique. The volume of urine from the left kidney was calculated gravimetrically, and the urine flow rate per Gram of each kidney weight (V′ u/ml/min gKW) was determined. Also, Cr clearance as an estimation of glomerular filtration
rate (GFR) [22], absolute excretion of Na (UNaV°), absolute excretion of K (UKV°), fractional excretion of K (FEK), and fractional excretion of Na (FENa) were determined by standard formulae.

3. Oxidative Stress Parameters

3.1. Malondialdehyde (MDA). The MDA assay was taken from Ohkawa [23] and adapted for our purpose. Briefly, 250 μl of tissue homogenate was suspended in 1000 μl of the reagent with 15% w/v TCA, 0.25 N HCl, and 0.375% w/v TBA. The solution was heated in a boiling water bath for 30 min. After centrifugation for 10 min at 3500 × g, the absorption was measured at 535 nm.

3.2. Protein Carbonyl (PCO) Content. The PCO level of plasma and tissue samples was assayed [24]. PCO was measured using the interaction between DNPH and the carbonyl groups to produce the yellow color that absorbs extremely at 370 nm. PCO content was determined utilizing a molar absorption coefficient (2.2 × 10^4 M⁻¹·cm⁻¹).

3.3. Total Thiol Content (tSH). Tissue homogenate and plasma total tSH were determined by the spectrophotometric method with slight modifications [25]. Briefly, 25 μl of tissue supernatant, 150 μl of the Tris-EDTA buffer, 790 μl of absolute methanol, and 10 μl of 10 mM DTNB were mixed in a 1.5 ml microtube. The absorbance was measured at 412 nm and the tSH level was determined using the molar absorption coefficient (13,600 M⁻¹·cm⁻¹).

3.4. Ferric Reducing Antioxidant Power (FRAP). This method was based on the ability of tissue homogenate in the regeneration of ferric ion (Fe³⁺) to ferrous ion (Fe²⁺) in the presence of tripyridyl s triazine (TPTZ) which was used as a reagent. The result was a blue complex TPTZ-Fe²⁺ with maximum absorption of 593 nm. FeSO₄ 7H₂O (0–1000 μmol/L) was used as the standard [26].

3.5. Antioxidant Enzyme Activities. The activities of antioxidant enzymes including superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase (CAT) in a homogenized renal tissue were determined using ELISA kits (ZellBio GmbH, Ulm, Germany) based on the kit guidelines.

3.6. Histological Evaluation. For histological assessment, kidney tissue samples were taken after blood collecting and were fixed in 10% formalin solution for one week. After embedding in paraffin, the tissues were cut into 3-4 μm sections. Then, the sections were mounted on the glass slides, stained with hematoxylin-eosin (H&E) reagent, and finally surveyed by a pathologist in a blinded way.

3.7. Statistical Analysis. Results were assessed utilizing a one-way ANOVA test. Tukey’s multiple comparison was utilized to determine statistical significance. The data were demonstrated as mean and standard error of mean (SEM). P ≤ 0.05 level was considered in all experiments.

4. Results

4.1. Biochemical Markers. Plasma Cr and urea were markedly augmented in I/R untreated group in comparison to the sham group (P < 0.001), while the treatment with OM extract, CAR, and Vit E significantly decreased these markers (P < 0.001) (Figures 1(a) and 1(b)). GFR was reduced in the I/R untreated group when compared to sham rats (P < 0.001), whereas treatment with OM extract and Vit E considerably increased it (P < 0.001) (Figure 1(c)).

The urine flow rate was insignificantly decreased in I/R untreated group as compared to the sham group; however, treatment with OM extract and its ingredients had no effect on it (Table 1). The UNaV° and UKV°, as well as fractional excretion of them (FENa and FEK), were markedly enlarged in the I/R group in comparison to the sham group (P < 0.001). Treatment with OM extract, CAR, and Vit E significantly decreased FENa and FEK as compared to untreated IR group (P < 0.001). However, only OM extract was able to reduce UNaV° (Figures 2(a) and 2(b), and Table 1). Urine Na and K were meaningfully augmented in I/R untreated rats as compared with the sham group (P < 0.001), while the treatment with OM extract (only UNa) and CAR markedly decreased these markers (P < 0.01) (Table 1).

Table 1. Effect of OM extract, CAR, and Vit E on some biochemical markers following I/R injury. Data are expressed as mean ± SEM. ** Significant differences with the sham group (P < 0.001), *** Significant differences with the sham group (P < 0.01), * Significant differences with the sham group (P < 0.05), ** Significant differences with the I/R group (P < 0.001), ** Significant differences with the I/R group (P < 0.01), *** Significant differences with the I/R group (P < 0.05).

4.2. Oxidative Stress Markers. The tissue FRAP and tSH contents were markedly reduced in the I/R untreated group in comparison to the sham group (P < 0.05). The administration of OM extract and Vit E significantly augmented tissue FRAP and tSH levels as compared to merely the I/R group, while treatment with CAR was able to increase the tissue FRAP levels in comparison with the I/R group (P < 0.01) (Figures 3(a) and 3(b)). The plasma levels of FRAP and tSH did not show a significant change in the I/R group in contrast to the sham group (Figures 4(a) and 4(b)). The plasma levels of PCO were markedly augmented in the I/R group as compared to the sham group, whereas treatment with OM extract significantly reduced it as compared to the I/R group (P < 0.05) (Figure 4(c)).

The tissue level of MDA was insignificantly enlarged in the I/R group in contrast with the sham group (51.92 ± 7.6
vs. 30.7 ± 4.6), while administration of OM extract, CAR, and Vit E slightly reduced it as against the I/R group (Figure 3(c)).

The CAT and SOD activity did no significant modification in the treated group in contrast with the I/R group (Figures 5(a) and 5(b)). The GPX activity was slightly reduced in the I/R group as against the sham group, while treatment with OM extract and CAR markedly increased it as compared to the I/R group (Figure 5(c)).

4.3. Histopathological Studies. Renal sections of the sham group showed normal morphology (Figure 6(a)). However, renal tissue of the I/R group significantly demonstrated
severe damage such as tubular necrosis (including hemorrhage, tubular dilation, and cytoplasmic vacuole formation), vascular congestion, and white blood cells (WBCs) infiltration as compared to the sham group ($P < 0.001$). Treatment with OM extract, CAR, and Vit E significantly decreased tubular necrosis, vascular congestion,
and lymphocyte infiltration in comparison to the I/R group ($P < 0.001$) (Figures 6(b)–6(e) and Figure 7).

5. Discussion

In the current study, we explored the impacts of OM extract, CAR, and Vit E on functional and histopathological changes induced by 30-minute kidney ischemia tracked by 24 h reperfusion in the right kidney of nephrectomized rats. In this study, mean arterial pressure did not reveal a significant change in different groups (data not showed), which indicates that modifications in renal function tests have not been due to changes in arterial pressure. Our findings indicated that I/R significantly reduced renal function by an increase in plasma Cr, and urea as well as a decrease in GFR. This increment in plasma Cr and urea [27, 28] and the drop in GFR are consistent with the other studies [29]. The increment in plasma Cr, urea, and decrement in GFR after I/R may be a result of vascular and tubular damage induced by oxidative stress [30]. In agreement with biochemical markers, renal I/R-induced typical morphological alterations, such as tubular necrosis, vascular congestion, and WBC infiltration. Treatment with OM extract, CAR, and Vit E significantly decreased plasma Cr and urea, increased
GFR, and caused a significant reversion of pathological changes. Amelioration of renal function in the current study might be a result of the antioxidant activity of OM extract, CAR, and Vit E. Previous studies indicated that OM extract had antioxidant activity. Also, it was able to inactivate hydroxyl radicals and hydrogen peroxide and inhibit lipid peroxidation [31–33].

**Figure 7:** Effect of OM extract, CAR, and Vit E on histopathological changes following I/R injury. (a) Tubular necrosis, (b) vascular congestion, and (c) white blood cells infiltration. Data are expressed as mean ± SEM. **∗∗∗**Significant differences with the sham group (P < 0.001). **###**Significant differences with the I/R group (P < 0.001). OM: *Origanum majorana*, I/R: ischemia-reperfusion, CAR: carvacrol, and Vit E: vitamin E.

Absolute excretion of Na and K were significantly increased in the I/R untreated group in comparison to the sham group, while treatment with OM extract only decreased UNaV. Furthermore, fractional excretion of Na and K were markedly augmented in the I/R group, and treatment with OM extract, CAR, and Vit E noticeably reduced these parameters. Despite the greatly reduced GFR in this study, the significant increase in UNaV and UKV are probably attributable to defect in Na and K reabsorption mechanisms, especially in the proximal tubule induced by I/R, which would be reflected as elevated in FENa and FEK.

Thiol groups are proper indicators of oxidative stress that defense against ROS. They have two types: glutathione (GSH) and protein thiol groups (PSH). There is a trivial alteration among the contents of tSH and PSH levels, as a result of the trivial amount of GSH [34]. FRAP is an estimation of the antioxidant storage in the insoluble phase. Consistent with a previous study [11, 35], after the I/R surgery, kidney FRAP, GSH, and tSH contents in the untreated I/R group were markedly reduced in contrast with the sham group. Low contents of FRAP and tSH in the kidney of I/R rats may be due to lower levels of antioxidant storage and higher depletion of GSH. GPX is a family of tetrameric enzymes that comprise selenocysteine amino acid within the active site. It is a free radical scavenger enzyme that consumes GSH and neutralizes ROSs such as lipid peroxides and H2O2 [36]. After I/R surgery, GPX activity was reduced [35] maybe as a result of an increase in the creation of ROSs. Our results showed that the FRAP and TSH levels, as well as GPX activity in the kidney tissue of the treated group with OM extract, were significantly augmented rather than the I/R untreated group. Altogether, the administration of OM extract (300 mg/kg) increased antioxidant capacity which was related to its high antioxidant character as a free radical scavenger. It seems that OM extract has more antioxidant power than CAR and Vit E, because it has more effect on tSH level and GPX activity.

Malondialdehyde, as an index of lipid peroxidation, had a longer half-life rather than ROS and could distribute to other areas to increase oxidative stress [37]. Topdagı et al. revealed that the values of tissue MDA were augmented in the I/R rats [38]. In comparison with this finding, our results showed that the MDA content insignificantly enlarged in the I/R group in contrast to the sham group. PCO formation is an early marker of protein oxidation that is mediated by free radicals [39]. According to Karaman et al. [40] study, oxidative damage of proteins occurs in I/R- induced kidney injury. This study showed that the plasma PCO level in the I/R untreated rats is markedly augmented in contrast with the sham group, while only OM extract (at a dose of 300 mg/kg) considerably reduced it. We conclude that treatment with OM extract merely was able to completely reserve protein oxidation and partially inhibit lipid peroxidation.

Oxidative stress has a key role in the pathogenesis of I/R injuries, such as tubular, and glomerular damage, that its injuries resulted in renal dysfunction [41]. The amelioration of oxidative stress indices and renal function (reduced Cr, and urea, plus increased GFR) in the present study showed that oxidative stress has a vital role in kidney dysfunction induced by I/R and OM extract or its ingredient, CAR, and Vit E have protective impacts against I/R- induced kidney injury.

**6. Conclusion**

We conclude that OM extract or CAR exerts renoprotective impacts in I/R-induced kidney injury possibly by ameliorating renal function markers, inhibiting protein oxidation, and renovating of ferric reducing antioxidant power, total thiol level, and GPX activity. The renoprotective impact of OM extract or its ingredient, CAR, seems to be related to trapping of free radicals and restoring of antioxidant power. Our findings suggest that the use of OM extract, as a

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supplementary therapy, could impede nephrotoxicity induced by I/R.

**Data Availability**

The data used to support the findings of study can be available at reasonable request to the corresponding author.

**Conflicts of Interest**

The authors declare there are no conflicts of interest.

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**References**


