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Ameliorative impacts of floral extract of *Salvia* species on oxidative stress and inflammation in rats renal ischemia/reperfusion

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I/R injury is a potentially serious problem that is encountered during a variety of medical and surgical procedures, such as thrombolytic therapy, organ transplantation and coronary angioplasty, The basic pathophysiology of I/R injury is microvascular dysfunction which is developed following reperfusion of ischemic tissues. It has clinical importance because of its frequent occurrence and mortality in some surgical conditions such as renal transplantation. Here, we investigated the protective effect of *Salvia* extracts on kidneys against I/R injury. Forty Spraque Dawley rats were divided into 5 groups. Right nephrectomy was performed to all groups. Gr. I, control; Gr. II, I/R; Gr. III & IV, I/R+50 and I/R+100 mg/kg *Salvia* floral extract; and Gr. V with I/R+50 mg/kg Rosmarinic acid. *Salvia* and Rosmarinic acid for 7 days was given single dose as a gavage.60 min ischemia, 60 min reperfusion were applied to groups except control. Intracardiac blood samples were taken, Blood urea nitrogen, creatine, malondialdehyde, myeloperoxidase, nitric oxide and chitotriosidase levels were detected. Mean values were evaluated by statistical analysis. The renal tissues were examined under light microscopy. Based on our biochemical and histological data, *Salvia* floral extract has potent anti-inflammatory and antioxidant effects against renal structure and function.

Keywords: Antioxidant activity, I/R injury, Kidney, Rosmarinic acid, Sage

Ischemia-reperfusion (I/R) injury often results in free radical formation [reactive oxygen species (ROS)] and inflammation within hours. Excessive production of oxidants and proinflammatory mediators leads to multiple organ dysfunctions[1]. Renal I/R injury can consist of some important clinical case such as partial nephrectomy, severe hypotension and subsequent resuscitation, kidney transplantation that can causative acute renal failure (ARF). The most common cause of ARF is renal ischemia, which causes renal functional impairment through a combination of renal vasoconstriction, renal tubular obstruction, tubular back leakage of glomerular filtrate, and decreased glomerular permeability[2]. Reactive oxygen species play a key role in the induction of kidney injury during I/R[3]. ROS, which are produced during renal reperfusion, directly damage cellular membranes through lipid peroxidation. Also, I/R injury causes endothelial dysfunction and local inflammatory responses to the kidney[4].

Nitric oxide (NO), a soluble, free radical gas, has an amazing range of biological roles, including modulation of vascular tone and inflammation. NO appear to play an important role during renal I/R injury. Furthermore, there is reciprocally between lipid peroxidation and oxidative stress. Lipid peroxidation is a catalytic mechanism leading to oxidative degradation of cellular membranes. Lipid peroxidation related to IR injury-induced tissue damage and malondialdehyde (MDA) is an indicator of the rate of lipid peroxidation[5]. At the same time in this study, myeloperoxidase (MPO) activity was used to measure the extent of inflammation and as an indicator of neutrophils accumulation.

The effects of *Salvia* on chitotriosidase activity using ischemia/reperfusion rat model has not been studied until now. In this context, our current study, i.e., chitotriosidase activity as the marker of inflammation gains originality.

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Recently, there has been an increase in consumer interest in using nutraceutical and functional foods worldwide[6]. Now, plant extracts have emerged on the market as antioxidants. The antioxidant capacity of these compounds has been proved to be comparison, and higher than, that of synthetic antioxidants. Natural antioxidants in foods are made polyphenolic compounds that can be found in all plant parts. Flavonoids are polyphenolic compounds with various pharmacological properties and act as scavengers of free radicals by OH groups in their molecular structure. Especially, the Lamiaceae family includes a large number of plants that are well known for their antioxidant properties[7]. Phenolic acids are widely distributed in the plantae, especially in the Labiaceae families. These compounds include caffeic acid monomers as well as oligomers of danshensu, caffeic acid, salvianolic acids, rosmarinic acid (RA), and lithospermic acid[8].

The genus *Salvia* (Lamiaceae) includes nearly 900 species spread throughout the world. This genus is represented in Turkey by 89 species and, altogether, 94 taxa, 45 of which are endemic in Turkey. The rate of endemism in the genus *Salvia* in Turkey is ca. 45%[8]. Salvia species are known as "adaçayı" in Turkey where they grow and consumed as a hot drink[9]. Many *Salvia* species are used as herbal tea and for food flavoring, as well as in cosmetics, perfumery and the pharmaceutical industries throughout world[10]. An infusion of aerial parts of *Salvia* spp. is used as a tonic, carminative, antiseptic, spasmolytic, astringent, hemostatic and diuretic as well as other therapeutic effects while the essential oil produced from this plant[11]. Essential oils and plant extract of some *Salvia* species, are believed to exert antioxidant, anti-microbial, antifungal, anti-inflammatory, carminative, diuretic, hemostatic, spasmolytic, and aromatic effects[12]. A well known medicinal plant *Radix Salvia miltiorrhiza* ('*Dansham*' in Korean and '*Danshen*' in Chinese), the root of *Salvia miltiorrhiza* Bunge (Labiatae), has been used in Chinese folk medicine for the treatment of coronary heart diseases, renal diseases, myocardial infarction, and hypertension[2].

In this study, we used ischemia/reperfusion rat model to investigate the effects of floral extract of *Salvia* upon inflammatory/oxidant process and share our knowledge about its *in vivo* effects.

Materials and Methods

Reagents

Salvia extract was supplied by Anadolu University, Faculty of Pharmacy, Department of Pharmacognosy (Eskisehir, Turkey). The flowering aerial parts of the *Salvia* species to be used in the study were powdered after drying with the appropriate method and the extraction method was used for the drug[13]. Rosmarinic acid (RA) was purchased from Sigma Aldrich (St Louis, MO, USA) All other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Animals experimental protocol

Forty adult male Sprague–Dawley rats weighting 250 to 300 g were obtained from TICAM (Medical and Surgical Experimental Research Centre, Eskisehir, Turkey) and housed in polycarbonate cages in a room with controlled temperature (25±2°C), humidity (50±5%), and a 12-h light/dark cycle. Animals had access to food and water ad libitum and were allowed to adapt to the local environment for one week before study. Feed and water were provided *ad libitum*.

All experimental studies were achieved in the Department of Medical Biochemistry, Faculty of Medicine, Eskisehir Osmangazi University, and the experimental design and procedures were approved by the Institutional Ethical Committee for Animal Care and Use at the Eskisehir Osmangazi University, Eskisehir, Turkey (Protocol No: 346/2013).

Rats were divided into five groups (each group n=8) randomly. Gr. I was designed as the control group. Rats in this group had access to food and water *ad libitum* during experimental period (7 days). Gr. II (renal I/R) was designed to show the morphological changes in the kidney after renal I/ R injury. Gr. III (renal I/R injury + 50 mg/kg *Salvia*), Gr. IV (renal I/R injury + 100 mg/kg *Salvia*) and Gr. V (renal I/R injury + 50 mg/kg RA) were designed to evaluate of *Salvia* on the morphological changes in the rats kidney in renal I/R injury. In our experiment, two different doses of *Salvia* (50 mg/kg, 100 mg/kg) and a single dose of 50 mg/kg *RA* given to rats by gavage were determined as a result of literature reviews[2]. The experiment was performed after a stabilization period in the laboratory for seven days.

Surgical procedures

Right nephrectomies

Right nephrectomies were performed under 2 mg/kg xylazine hydrochloride (Rompun, Bayer) and

50 mg/kg ketamine hydrochloride (Ketalar, Pfizer) anesthesia in all rats in all groups (I–V).

Drug administration

Prior to the left nephrectomies, 50 and 100 mg/kg *Salvia* were administered for 7 days to the rats in Groups III and IV respectively. Also, 50 mg/kg RA was administered for 7 days to the rats group V. All tested solutions were administered orally (oral gavage) once a day at 9:00 a.m. for 6 days.

Induction of renal I/R injury

Renal I/R injury were induced with left renal pedicle occlusion with a vascular clamp for 60 min, followed by reperfusion for 60 min through a median laparotomy under anesthesia. Procedure of control was the same beyond vascular occlusion in Gr. I. after induction of I/R injury in Gr. II, III, IV and V left nephrectomies were performed for histopathological examinations under additional ketamine anesthesia. Rats in Gr. I underwent left nephrectomy 60 min after the control procedure. Blood samples were collected at 60 min after reperfusion and the rats were sacrificed afterwards while the kidney tissue was harvested[14].

Biochemical analysis

Blood samples were centrifuged at 1097 ×g for 10 min (Jouan MR 22). Blood Urea Nitrogen (BUN), Creatinine (sCR) were immediately measured with Roche Diagnostic kits in a Modular Systems (Roche Diagnostics) analyzer according to the manufacturer's instructions.

Malondialdehyde (*MDA*) *measurement*

Determination of tissue lipid peroxidation was assayed by quantifying MDA in the form of thiobarbituric acid reaction according to the method of Ohkawa *et al*.[15]. Frozen kidney samples were weighed and homogenized in ice-cold 0.15 M KCl buffer (1%, 15 w/v, pH 7.4) using a homogenizer (Ultra Turrax 125-Janke Kunkol; United Kingdom). After centrifugation at 1000 rpm for 15 min, the supernatants were collected.

Absorbance of the pink-coloured thiobarbituric acid reactive substances were spectrophotometrically measured at 532 nm (UV-1601; Shimadzu, Tokyo, Japan). The total protein contents of the samples were calculated using the Biuret method[16]. The MDA levels were expressed in nano moles per miligram.

Myeloperoxidase (*MPO*) *measurement*

Determination of tissue myeloperoxidase activity was assayed according to the method described by Suzuki *et al*.[17]. The samples were weighed and homogenized in 1:10 (w/v) 50 mM potassium phosphate buffer (pH 7.4) using the same homogenizer device.

The homogenates were centrifuged at 15.000 ×g for 10 min at 4°C, and the supernatants were resuspended in an equal volume of detergent-containing buffer (50 mM potassium phosphate Buffer pH 6, 0.5% HETAB, 10 mM EDTA). The reaction started by the addition of 50 µL of H_2O_2 (diluted to 0.06%) at 37°C. The rate of MPO-catalyzed oxidation of tetramethylbenzidine was followed by recording the increase of absorbance at 655 nm using spectrophotometry. The enzyme activity was expressed as the amount of enzyme producing one absorbance change per minute under assay conditions in units per gram tissue. The results were manipulated after the protein contents of the samples were calculated using Biuret method[16].

Total Nitrite (*NO2* $^-$) *levels measurement*

Frozen kidney samples were weighed and homogenized in ice-cold 1 M phosphate buffer (pH 7). Homogenates were centrifuged at 600 ×g for 10 min at 4°C. Nitrate and nitrite, which are totally reflects the best index of total NO production, are the final and stable end products of NO *in vivo* Nitrate was assayed by modification of the cadmium-reduction method as described by Cortas and Wakid[18]. The nitrite produced was determined by diazotization of sulfanilamide and coupling to naphthylethylene-diamine. After the samples were deproteinized nitrate was reduced by copper coated cadmium in glycine buffer at pH 9.7 (2.5-3 g cadmium granules for a 4-mL reaction mixture). The reduction followed pseudo-first-order reaction kinetics; a convenient time interval for the assay was 90 min. The maximum reduction occurred at approximately 2 h. The results were expressed as nanomoles per milligram protein after the protein contents of the samples were calculated using Biuret method[16].

Chitotriosidase (*ChT*) *enzyme assay*

Chitotriosidase enzyme assay was based on the method described by Hollak et al. with minor modifications[19]. Briefly, chitotriosidase activity was determined by incubating 10 µL of serum with 200 µL of 22 µmol/L fluorogenic substrate 4-methylumbelliferyl-β-D-N,N',NN'-triacetylchitotrioside (Sigma) in McIlvain buffer (100 mmol/L citric acid and 200 mmol/L sodium phosphate, pH 5.2) for 15 min at 37°C. The reaction was stopped with 2 mL of 0.3 mol/L

glycine-NaOH buffer (pH 10.6) by mixing at room temperature.

The substrate hydrolysis by chitotriosidase produces the fluorescent molecule 4- methylumbelliferone, which was quantified with a fluorometer, excitation at 366 nm and emission at 446 nm, and compared with a Standard 4-methylumbelliferone calibration curve. Chitotriosidase activity was expressed as nanomoles of substrate hydrolyzed per hour per milliliter of incubated serum.

Histological analysis

Light microscopy. Left nephrectomy specimens were processed routinely in 10% formalin solution and embedded in paraffin. Tissue sections of 5 µm were obtained and stained with hematoxylin and eosin (H&E). Histopathological examinations were performed under a light microscope at 10X and 40X magnification, which was connected to a computer. All specimens were examined for the existence of focal glomerular necrosis, bowman capsule dilatation, tubular epithelial degeneration, tubular epithelial necrosis, tubular dilatation, interstitial inflammatory infiltration, and peritubular congestion. A minimum of ten fields for each kidney slide with a minimum of magnification was examined to assign the severity of these morphological changes. The morphological changes were scored (0: absent; 1: mild; 2: moderate; 3: severe) in order to perform a comparison between the groups.

Statistical analysis

Data analyses were performed using IBMSPSS version 21 (IBM Global Services Limited Company, Istanbul, Turkey) and Sigma Stat 3.5 software packages (Systat Software Inc, San Jose, CA). Shapiro-Wilk test of normality was used to assess compliance with the normal distribution of continuous variables in each group. Continuous normally distributed measurements were compared across the groups using one-way analysis of variance with the Tukey method for multiple comparisons. Measurements that did not demonstrate a normal distribution were compared using the Kruskal-Wallis (for independent samples). Results were presented as means±SD (standard deviation) and medians (25-75%). The significance level was established at P <0.05.

Results

Renal function parameters

The blood urea nitrogen and serum creatinine levels in the I/R group were found to be significantly higher than those in the control group rats (P <0.001; Table 1). Both 50 mg/kg *Salvia* and 50 mg/kg RA pretreatment significantly decreased sCR and BUN concentrations compared with the I/R (P <0.05). Also 100 mg/kg *Salvia* was better than 50 mg/kg *Salvia* and 50 mg/kg RA. These results imply this experimental process by rats was enough to obtain ischemia/ reperfusion model.

Marker of oxidative stress tissue

Tissue MDA levels

I/R process was increased the level of lipid peroxidation (MDA content). MDA levels of control and all treatment groups were significantly lower compared to I/R group (P <0.001) (Fig. 1). There was an increase in the MDA levels after I/R compared with the control rats (P <0.001). Significant decreases resulted in MDA levels of 50 mg/kg *Salvia* (P <0.05), 100 mg/kg *Salvia* (P <0.001), 50 mg/kg RA respectively. However, no significant difference was found among the *Salvia* groups. Also, 50 mg/kg *Salvia* and 50 mg/kg RA therapies didn't decrease

Table 1 — Effect of *Salvia* extract pretreatment on sCR and BUN levels in experimental rats

Experimental Groups (n=8)	BUN Median (%25-%75)	sCR Median (%25-75)
Gr. I (control)	24,45 (23,45-25,77)	0,37 (0,35-0,39)
Gr. II	42,70 (38,25-47,00)[a]	0,70 (0,65-0,72)[a]
Gr. III	40,65 (39,97-40,97)[a,c]	0,72 (0,67-0,74)[a,c]
Gr. IV	29,70 (27,97-31,17)[b]	0,61 (0,49-0,68)[d]
Gr. V	40,15 (37,75-42,07)	0,60d (0,58-0,66)[a]

[The results are presented median (25–75 % percentile) for each group. [a]Significance against Gr. I (control) values: P <0,001; [b]Significance against Gr. II values: P <0,001; [c]Significance against Gr. II values: P <0,05; and [d]Significance against control values: P <0,05. The levels in terms of statistical significant were evaluated with Kruskall–Wallis 1- way ANOVA]

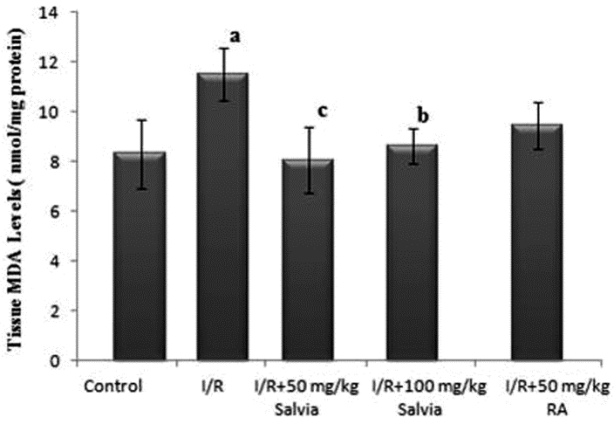

Fig. 1 — Comparison between groups of tissue MDA levels. [Results are given as mean±SD for each group. [a]Significance against Gr. I (control) values: P <0,001; [b]Significance against Gr. II values: P <0,001; and [c]Significance against Gr. II values: P <0,05]

MDA levels as much as the Gr. IV (100 mg/kg *Salvia*) therapy did (P <0.001).

The protective role of *Salvia* against lipid peroxidation was increased by dose dependent manner. 100 mg/kg *Salvia* had better effects than 50 mg/kg *Salvia* and 50 mg/kg *R*A against lipid peroxides (Fig. 1).

Markers of Inflammation

Tissue MPO levels

The MPO activity increased significantly in the I/R group compared with the control (P <0.001). Significant decreases in MPO activities were observed in all *Salvia* groups. The lowest MPO activity was obtained in the 100 mg/kg *Salvia* group followed by the 50 mg/kg RA, 50 mg/kg *Salvia* groups (Fig. 2).

Leukocyte, especially neutrophil, infiltration to the kidney also involves in renal IR. We investigated MPO activity, a marker of neutrophil infiltration, in the kidney and found that renal IR produced marked increase in MPO activity, indicating that neutrophils infiltration into kidney tissue was more emphasize compared with control animals. The values of tissue MPO levels are shown in Fig 2. The data indicated that the renal ischemia (Gr. II) had significantly increased tissue MPO levels when compared with the control group (P <0.001). Furthermore, the pretreatment with 100 mg/ kg *Salvia* significantly protected renal function in the rats subjected to renal ischemia (P <0.001).

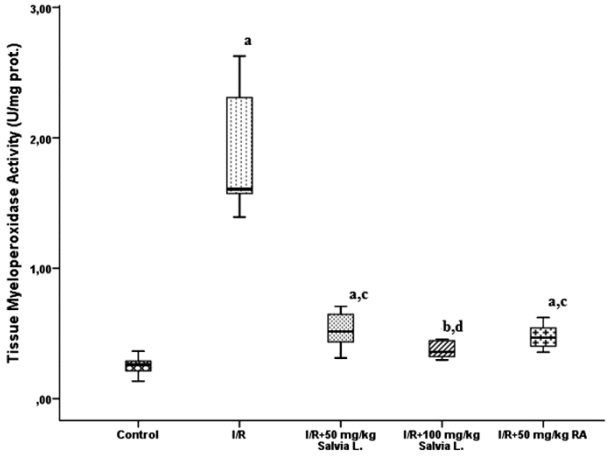

Fig. 2 — Tissue MPO activity (U/mg protein) presented median (25-75% percentile) for each group. [[a]Significance against Gr. I (control) values: P <0,001; [b]Significance against Gr. II values: P <0,001; [c]Significance against Gr. II values: P <0,05; and [d]Significance against Gr. V values P <0,05. The levels in terms of statistical significant were evaluated with Kruskall–Wallis1-way ANOVA]

Tissue NO levels

Fig. 3 shows the tissue NO levels in all groups. There was a decrease in the NO levels after I/R compared with the control rats (P <0.001). Significant increases resulted in NO levels of 50 mg/kg *Salvia* (P <0.05), 100 mg/kg *Salvia* (P <0.001), 50 mg/kg RA, respectively. The NO level was significantly lower in the I/R group than in the control group (P <0.001), while it was markedly elevated (P <0.001) in the 100 mg/kg *Salvia* pre-treatment groups compared to the I/R group (P <0.001).

Chitotriosidase activity

Serum chitotriosidase activity is increased in renal ischemia reperfusion injury. Chitotriosidase, quantitative proteins secreted by activated macrophages, is a human chitinase member of family 18 glycosyl hydrolases. Its activity has been proposed as a biochemical marker of macrophage accumulation in several lysosomal diseases, especially in Gaucher's disease[19]. Differences were statistically significant between I/R versus control group (P <0.001) and between I/R group (Gr. II) *vs*. 100 mg/kg *Salvia* group (Gr. IV) (P <0.001). Also, statistically significant between I/R group (Gr. II) *vs*. 50 mg/kg *Salvia* group (Gr. III) (P <0,05) (Fig. 4).

Histologic results

Histologic changes according to the groups are shown in Fig. 5. The control group exhibited normal renal morphology, whereas the other groups detected differential degrees of tissue damage. Severe tissue

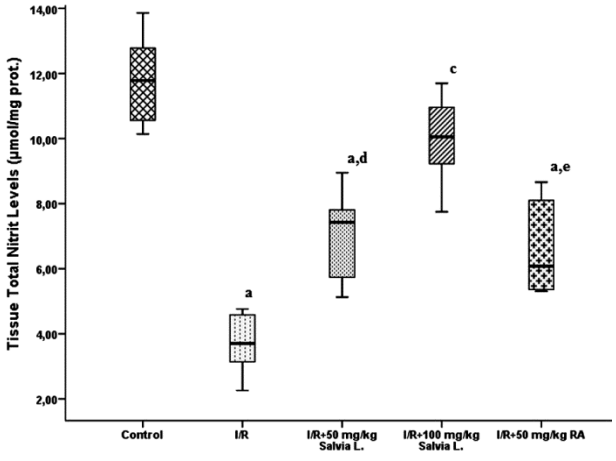

Fig. 3 — Tissue total nitrite levels (µmol/mg protein) presented median (25-75% percentile) for each group. [[a]Significance against Gr. I (control) values: P <0,001; [b]Significance against Gr. II values: P <0,001; [c]Significance against Gr. II values: P <0,001; [d]Significance against Gr. II values: P <0,05; [e]Significance against Gr. IV values: P <0,05; and [f]Significance against Gr. V values P <0,05. The levels in terms of statistical significant were evaluated with Kruskall–Wallis-way ANOVA]

destruction was observed in the I/R groups. This was significantly higher compared with all the *Salvia* groups (P <0.001). The lowest damage score was viewed the 100 mg/kg *Salvia* group. The median (min–max) scores of the morphological changes in the groups are summarized in Table 2. In Gr. II (renal I/R injury), significant focal glomerular necrosis, Bowman capsule dilatation, tubular epithelial degeneration, tubular epithelial necrosis, tubular dilatation, interstitial inflammatory infiltration, and peritubular congestion were observed due to renal I/R injury (Table 2). In Gr. IV, in which the rats were treated with 100 mg/kg *Salvia* floral extract before renal I/R, tubular epithelial degeneration, tubular epithelial necrosis, tubular dilatation interstitial inflammatory infiltration and peritubular congestion were observed to be significantly attenuated with the treatment (Table 2 and Fig. 5).

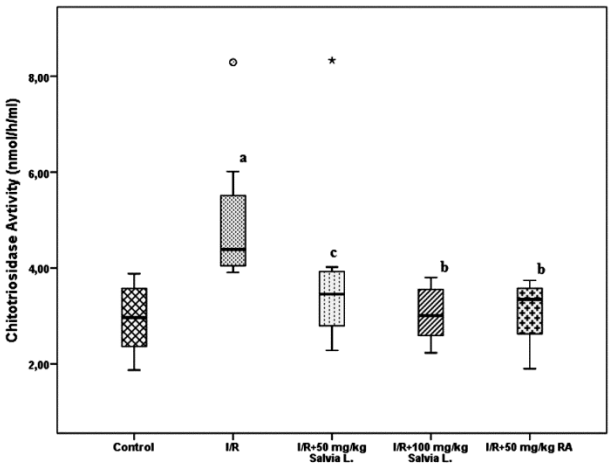

Fig. 4 — Chitotriosidase activity (ChT; nmol/h/(mL) levels in serum. The results are presented as median (25–75% percentile) for each group. [a]Significance against Gr. I (control) values: P <0,001; [b]Significance against Gr. II values: P <0,001; and [c]Significance against Gr. II values: P <0,05. The levels in terms of statistical significant were evaluated with Kruskall–Wallis 1- way ANOVA]

Dıscussıon

Observed changes in renal function are common in numerous diseases. Despite the advances in recent years, mortality and morbidity rates remain high, reaching 60%. In the renal formation, acute tubular necrosis accounts for 75% of the cases of acute kidney injury (AKI)[20].

Renal ischemia is a widespread problem during kidney transplantation, partial nephrectomy, cardiopulmonary bypass, or hydronephrosis leading to renal dysfunction and injury[5]. Ischemia is an irreversible tissue injury process. This process was defined as a loss of blood supply in a tissue due to

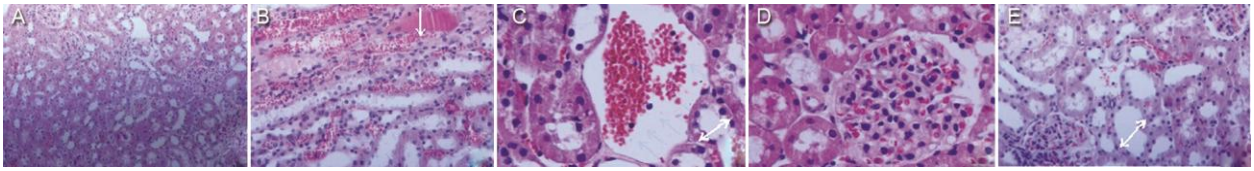

Fig. 5 — Morphological images of groups. (A) Normal morphology in Gr. I. (scale bar: 200 μm); (B) Acute tubular necrosis in Gr. II. (scale bar: 100 μm); (C) Tubular dilatation in Gr. III with 50 mg/kg *Salvia* flower extract before renal I/R; (D) Very mild congestion in Gr. IV; and (E) Peritubular congestion, tubular dilatation in Gr. V]

Table 2 — Damage scores according to study groups

	Group I	Group II	Group III	Group IV	Group V
Focal Glomerular Necrosis	0,00 (0,00-0,00)	1,50 (1,00-2,00)***	1,00 (1,00-2,00)***,##	0,50 (0,00-1,00)	1,00 (1,00-2,00)***,##
Bowman Capsule Dilatation	0,00 (0,00-1,00)	2,00 (1,00-2,00)***	2,00 (1,00-2,00)***	1,00 (0,00-1,00)	2,00 (1,00-2,00)***
Tubular Epithelial Degeneration	0,00 (0,00-0,75)	2,00 (2,00-2,75)***,###	2,00 (2,00-2,00)***,###	0,00 (0,00-0,75)aaa	2,00 (2,00-2,00)***
Tubular Epithelial Necrosis	0,00 (0,00-0,75)	1,50 (1,00-2,00)***,#	2,00 (1,00-2,00)***,###	0,50 (0,00-1,00)a	1,00 (1,00-2,00)***
Tubular Dilatation	0,00 (0,00-1,00)	2,00 (1,25-2,00)***	2,00 (1,25-2,00)***,###	0,00 (0,00-0,00)aaa	2,00 (1,00-2,00)***,###
Interstitial Inflammatory Infiltration	0,00 (0,00-0,75)	2,00 (1,00-2,00)***	2,00 (1,00-2,00)***,###	0,00 (0,00-1,00)aaa	2,00 (1,00-2,00)***,###
Peritubular Congestion	0,00 (0,00-0,75)	2,50 (2,00-3,00)***	2,00 (2,00-2,75)***,###	1,00 (0,00-1,00)aaa	2,00 (2,00-2,00)***,###

[Damage scores 0: absent; 1: mild, 2: moderate, and 3: severe. *Significance against Gr. I (control) values: *P <0,05 and ***P <0,001; [a]Significance against Gr. II values: [a]P <0,05 and [aaa]P <0,001; [b]Significance against Gr. III values: [b]P <0,05 and [bbb]P <0,001; [#]Significance against Gr. IV values: [#]P <0,05 and [###]P <0,001]

prevent arterial flow or reduced venous drainage. It is associated with oxidative stress which is a result of the imbalance between the production of reactive oxygen species (ROS), antioxidants, and repair processes[21]. On the basis from this information was considered to prevent and block of renal ischemia reperfusion injury by using of some agents or drugs before any attempt to be made to the kidney. Precise time of ischemia is needed to reveal experimentally the effects of consisting of I/R damage in kidney. In this study similar studies were examined to indicate time of the ischemia-reperfusion period in the literature[22-26].

Obviously, new treatment protocols are necessary to understand damage of I/R better. On the other hand, before clinic trial, therapeutic drugs have to try and observe with animal trials according to human. Tested and developed antioxidant agents which is applied to clinical it takes long process but many antioxidant is existed in various pathological situations such as organ protective[27]. Many agents had been used to inhibit renal I/R injury during literature researching. The use of such materials by testing, its getting be interested about idea of which another materials would be effective in I/R injury.

In our study, extracts of *Salvia*, is widely used in traditional medicine worldwide. *Salvia* is reported to have a wide range of biological activities, including antimicrobial, sedative, anti-inflammatory, antioxidant, antitumor, antihypertensive, diuretic, antibacterial, fungi static, virustatic, astringent and antihydrotic effects[28]. Phytochemical analysis of the *Salvia* showed that it contains hydroxycinnamic acid derivatives (caffeic acid and rosmarinic acid), flavonoids (luteolin, apigenin and glicosides) and diterpenoids (carnosol, carnosol acid and methyl carnosoletc)[29]. In our study, we included Rosmarinic acid (RA) owing to most powerful flavonoid inside Species of *Salvia* so as to positive control. RA is a natural, phenol, antioxidant carboxylic acid. Also, RA in medicinal plants, herbs and spices, has been generated healthy and beneficial effects. Many bioactivities of RA have been reported, such as anti-liver fibrosis, antisepsis and anti-diabetic nephropathy. Among these were several RA biological activities, including antioxidant, astringent, anti-inflammatory, antimicrobial, antiangiogenic, antiviral, antirheumatic, antiallergic, antidepressant, antidiabetic, and antitumor activities of RA were also determined. RA was shown to inhibit superoxide anion production in the xanthine/xanthine oxidase system, thus being effective in protecting biological systems against oxidative stress. RA was also shown to act as a powerful inhibitor of lipid peroxidation in microsomal and liposomal systems, and to scavenge peroxyl radicals and hydrogen peroxide[30]. There are quite studies which were observed about *RA* have antioxidant, anti-inflammatory and antiallergenic activity. We evaluated protective effect of the *Salvia* because of these pharmacology properties on renal I/R injury and included in our study. *Salvia* extracts were used in our study. Because these plants are endemically grown in our city. After researching of literature indicated different two doses of this endemic species which were used[31].Chen et al. reported that the beneficial effects of pre-treatment with *Salvia miltiorrhiza* ethanol extracts on renal function markers, immunity and antioxidant activities in renal ischemia and reperfusion (IR) rats. They were subjected to 60 min of global ischemia at 37°C followed by 30 min of reperfusion. They animals were orally administered *Salvia miltiorrhiza* ethanol extract (50,100 or 150 mg/kg) or tanshinone (25 mg/kg) or a control vehicle (saline) daily for 20 days. Their effective dose was 100 mg/kg as same in ours[2].

The aim of this study was to detect the reduction with *Salvia* treatment of renal I/R injury-induced biochemically and histologically changes in the rat kidney. These effects were observed when *Salvia* was administered by gavage at doses of 50 and 100 mg/kg.

Our results have shown that renal I/R caused kidney damage, with an increase in the levels of sCR and BUN, and significant alterations in the histopathological features when compared to the control rats. Pretreatment with *Salvia* the renal injury. Furthermore, pre-treatment with RA improved renal functions, reduced the levels of sCR and BUN, and preserved the normal morphology of kidneys. We were determined that similar studies results were parallel with our findings[32-36].

Myeloperoxidase activity in kidneys was used as an indicator of polymorphnuclear leukocyte (PMN) infiltration into renal tissues. PMNs also release myeloperoxidase, which catalyses the formation of another potent oxidant, hypochlorous acid[37]. Therewithal, PMNs also generate cytokines and interact with the renal endothelium leading to further pathophysiology. Rats exposed to renal I/R exhibited

a substantial increase in kidney MPO activity, suggesting increased PMN infiltration into renal tissues. However, pretreatment of rats with *Salvia* and RA prior to I/R produced reduction of MPO activity to levels observed from control animals.

We found that *Salvia* in kidney significantly improved renal function and protected against renal damage caused by I/R injury, and these responses were associated with the effect of reducing proliferation of T and B lymphocyte. Also, leukocytes and mononuclear cell migration was significantly inhibit in their study.This was also shown by Tan *et al*.[38] and Qiao *et al.* [39] in which MPO activities increased less in I/R compared with controls. Our results were consistent with the findings obtained in their studies. On the other hand, in I/R injury, reperfusion of vibrant tissues in that organs after ischemia may aggravate cellular injury and tissue destruction. Ischemia is closely associated with oxidative stress. During oxidative stress, it is believed that an important cause of destruction and damage of cell membranes is lipid peroxidation mediated by ROS. MDA, a marker of tissue injury, is used as an indicator of lipid peroxidation[40]. In this study, pretreatment with *Salvia* showed a significant decrease in MDA production. Peroxynitrite is one of the sources of ROS during renal I/R. Several experiments have detected that renal ischemia is associated with lipid peroxidation, which is an autocatalytic mechanism causing oxidative destruction of cellular membranes, and this destruction can cause the production of reactive metabolites, toxicity and cell death. Lipid peroxidation, as a free radical generating system, has been proposed to be closely related to IR induced tissue injury and MDA is a good indicator of the degree of lipid peroxidation

Consistent with our findings, Ge et al. reported that *Salvia miltiorrhiza* significantly decreased the level of MDA in myocardium oxidative injury. Kang et al. also demonstrated that lithospermic acid B isolated from *Salvia miltiorrhiza* significantly decreased the level of MDA after ischemia/reperfusion-induced renal injury in rats. This protective effect of *Salvia* may be in part mediated by scavenging the very reactive $ONOO^-$ and OH[41,42]. Nitric oxide (NO), which is a potent endogenous vasodilator, is generated by NO synthase (NOS) in the endothelium. Several studies have shown that there is a decrease in NO levels in the renal cortex during the renal ischemia reperfusion.

In our study, we examined to determine the protective effect of *Salvia* on renal I/R injury and investigated the possible underlying mechanism of the effects in rats. Essential production of NO synthesis plays a major role of control the functions of the proximal convoluted tubule and for reabsorbing 65-70% of Na+, water reabsorption[43]. In our study, I/R caused a marked decline in the NO level. Pre-treatment with *Salvia* at doses of 50, 100 and RA at dose 50 mg/kg increased the NO content. NO has been indicated prevent from oxidative stress, cytokine release, adhesion and aggregation of neutrophil leukocytes[44]. Several studies have shown a relationship between NO and IR injury. A reduction of NO during IR is generally caused by endothelial dysfunction and reduction of endothelial nitric oxide synthase activity[45]. Our results were consistent with these findings. These findings showed that renal IR injury is associated with endothelial dysfunction and reduction of nitric oxide synthase activity. *Salvia* in IR induced remote liver injury, may exhibit its effect by increasing NO synthase activity and releasing NO from vascular endothelium. Pretreatment with *Salvia* enhanced the renal damage.

The another section which is recently drawn attention about new marker of inflammation Chitotriosidase's that provide originality of our study with examining in ischemia reperfusion model. Recently, Boot *et al*.[46] have shown that chitotriosidase activity was elevated up to 55-fold in extracts of atherosclerotic tissue, showing a clear connection between chitotriosidase expression and lipid-laden macrophages inside human atherosclerotic vessel wall. The serum chitotriosidase activity increases in ischemia/reperfusion injury. To evaluate this hypothesis, we analyzed the serum chitotriosidase activity and described chitotriosidase level in rats with I/R injury. We have used of plant extract in this study, increased level of ChT in I/R group without related any species was decreased relatively by *Salvia* The decline effect may be associated with plant extract's anti-inflammatory properties and blockage of macrophage activation. Moreover, after our literature researching, remarkable point that there is no studies about how ChT's active is shown effect against I/R injury.

In our study, when the kidney tissue was histologically examined, prominent findings such as

intense congestion, plasma exudations, hydrophic swelling in tubular cells, shedding of necrotized cells into the tubule lumen, and acute tubular necrosis observed in the I/R group. These structures in the 100 mg/kg *Salvia* administered group were replaced by significant improvement in basement membrane structures, normal appearance of tubular widths and tubular cells, minimal plasma exudations in the tubule, and normal monitoring of glomerular structure. According to the information we obtained; we consider that 100 mg/kg *Salvia* extract can largely eliminate I/R-induced renal damage.

Conclusion

In summary, based on our biochemical and histological data, serious damage was observed in rat tissue samples in terms of oxidation and inflammation as a result of renal damage induced by I/R. However, *Salvia* floral extract @50 and 100 mg/kg, and Rosmarinic acid @50 mg/kg demonstrated significant protective effect against kidney structure and functions. *Salvia* floral extract @100 mg/kg prevented renal tubular necrosis and thereby ameliorate I/R induced renal injury. These results indicate the scope using *Salvia* floral extract before surgical procedures to reduce oxidative renal tissue damage and prevent severe deterioration of renal function. However, clinical implementation of these findings requires further human experimental studies.

Conflict of Interest

Authors declare no competing interests.

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