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Research Article

Co-administration of hydroalcoholic *Viola odorata*'s extract and streptozotocin on histopathological and biochemical changes in rat kidney

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ABSTRACT

This study aimed to evaluate the effect of hydroalcoholic *Viola odorata* extract (VOE) on kidney tissue in a diabetic rat model induced by streptozotocin (STZ). Male Wistar rats were randomly divided into five groups: 1. Vehicle controls, 2. STZ, 3. STZ+ 100 mg/kg (VOE), 4. STZ+ 200mg/kg (VOE) 5. STZ+ 400 mg/kg (VOE). At the end of the experiment serum and urinary levels of urea, creatinine, albumin, serum glucose, and MDA levels were measured. Tuft glomerular and renal corpuscles area, epithelial height in proximal and distal convoluted tubules (PCT and DCT) were quantitatively measured in histologic slides. VOE in doses of 100, 200, and 400 mg/kg significantly reduced the serum glucose level. VOE in dose of 200 mg/kg reduced urine albumin, creatinine, and urea in comparison with STZ treated group (P<0.001). VOE in a dose of 400 mg/kg also reduced urine albumin (P<0.01). There were no significant statistical differences in serums' kidney markers between the STZ and VOE groups. 100 mg/kg and 200 mg/kg of VOE reduced serum MDA level compared to the STZ group. Treatment with various doses of VOE effectively reduced both the tuft glomerular and renal corpuscles area. Epithelial height in PCT and DCT reduced in STZ treated group compared to controls. 200 mg/kg VOE increased them significantly (P<0.001).The results showed that VOE has hypoglycemic properties. VOE may protect renal function induced by STZ, especially in a dose of 200 mg/kg and through reduction of oxidative stress.

1. INTRODUCTION

Diabetes is a metabolic disorder of the endocrine system[1]. Diabetes Mellitus (DM) is one of the five leading causes of death in the world[2]. The global prevalence of diabetes has been dramatic over the past 10 years[3]. The important causes of this disease are family history, poor dietary patterns, obesity, a high caloric diet, and sedentary behavior. Hyperglycemia leads to various organs dysfunction, such as kidneys, liver, heart, reproductive system and blood vessels[3]. Kidney function is impaired in DM that is characterized by proteinuria and loss of renal function. In the end-stage of DM hypertension is occurred. Histologically DM changes kidney structure including glomerular basement membrane, mesangial expansion, and also glomerulus-clerosis[4]. There is evidence that shows antioxidant enzymes are impaired in diabetes mellitus[5]. Therefore, it suggests oxidative stress has an important role in the development of DM[6]. Previous

studies in diabetic patients and diabetic animals have shown that free radicals, membrane lipid peroxidation, and protein oxidation are increased. Insulin and hypoglycemic agents are the main effective treatment for DM. Hypoglycemia, weight gain, headache, and elevating liver enzymes are the side effects of these agents[5].

The WHO has also recommended the use of herbal anti glycemic agents in traditional medicine[7]. Side effects of herbal drugs and plants are fewer in compare with chemical drugs. Researchers are looking for new herbal compounds for the treatment or preventing the disease[8]. Applicants for new herbal medicine are on the rise today. They hope that this new herbal medicine will reduce the side effects of chemical and synthetic drugs.

Viola odorata (V.odorata) is commonly known as sweet violet and is native to Europe and Asia, as well as in North America and Australia[9]. Different types of violets are used to improve liver and kidney function[10]. Studies have shown that some species of this plant are rich in antioxidants because of phenolic compounds[11].

This study aimed to investigate the effect of *V. odorata* on kidney structural changes, some renal function and serum oxidant status in diabetic rats.

2. MATERIALS AND METHODS

2.1. Preparation of extract

The flowers of *V.odorata* were collected from Ramsar, Mazandaran province in North of Iran, in May, 2017. The plant was identified and given herbarium specimen number (HGUM-302). The voucher specimen was deposited in herbarium of school of pharmacy, Guilan University of Medical Sciences, Rasht, Iran. The flowers (500 g) were shade dried and crushed to a powder and then extracted by percolation with ethanol (70%), at room temperature for one week. The solvent was evaporated by rotary evaporator and stored in a refrigerator until required[11].

2.2. Animals and induction of diabetes:

In this study, 40 adult Wistar male rats (250 ± 10 g) were used. Animals were randomly divided into five experimental groups. The rats were maintained under standard conditions with a 12-h light and 12-h dark cycle at room temperature (23℃). They had access to food and tap water ad libitum. This study was done by following the guidelines provided by the Animal Laboratory. All principles of animal work were carried out according to the ethics committee approved by Guilan University of Medical Sciences (animal protocol number: IR.GUMS.REC.1395.226).

Control group received normal saline, STZ group: received STZ 45 mg/kg, STZ groups were divided into three subgroups and treated with 100, 200, and 400 mg/kg *V. odorata* extract (VOE).

In this study to induce diabetes, a single dose of STZ (45 mg/kg) was used intraperitoneally. STZ solution prepared freshly and injected. After three days, blood glucose (BG) was measured. Animals with BG levels >250 mg/dl were considered as diabetic[12-13].

Administration of VOE started 3 days after induction of diabetes by the injection of STZ. VOE were administered intraperitoneally once a day, for 30 days. At the end of the experiment, animals were weighted and urine samples were obtained for the study of renal function. Fresh urine collected with a mild intervention as previously is described by Khosho et al in 1985[14]. Each mouse was placed in a cool glass finger bowl (diameter 16 cm) to stimulate urination. Urine samples were collected twice, 8-9 am and 13-14 pm in each mouse. Then both urine samples were pooled for analysis[15]. For collecting more urine sample the back of the animal was tickled using the fingers and also a cold pad was put below the animal's abdomen for about 30 seconds. Then using a Pasteur pipet urine collected in a 2ml vial and stored at 20°c until analysis. All surgery was performed under anesthesia and every effort was made to minimize suffering. Animals were anesthetized intraperitoneally with 50mg/kg ketamine and 22 mg/kg xylazine. The right kidney was removed for histologic study. A Blood sample obtained from inferior vena cava.

2.3. Biochemical assay

Blood samples were centrifuged at 4000 rpm for 10 min. Then serum separated and stored at -20˚C. FBG levels were measured using (Parse Azmun –Iran) kit. Serum and urinary, creatinine albumin and urea levels were measured by the kits were tested using a photometric method.

2.4. Malondialdehyde (MDA) Assay

Content of MDA assayed in serum using ELISA kit (Bioassay, China). The basis of the assay was redox colorimetric. After reaction optical absorption read at 450 nm wavelength.

The sensitivity of the kit was 0.14 nmol/ml and the diagnostic range was 0.2-60 nmol/ml.

2.5. Histologic study

All kidney tissues were fixed in 10% neutral buffered formalin for 72 h. Then the tissue passage was carried out with alcohol with graded doses of 50, 70, 90, 100% for dehydration, and xylene for infiltration, and paraffin for molding. Using a rotary microtome (Leitz - Germany), 5-micron sections were prepared. All observations were made by an optical microscope (Olympus - Japan) with 400×. At least 15 renal corpuscles/ animal and 15 tubules (proximal and distal) /animal evaluated quantitatively. Measurements, such as the area of the glomerular tuft and renal corpuscles, the epithelial height of the proximal convoluted tubule (PCT) and distal convoluted tubules (DCT) were studied. To evaluate the histomorphometry, the software Digimizer Version 11 was used and data were expressed as micrometer (µm) and square micrometer (μm^2).

2.6. Data analysis methods:

The distribution of data for normalization was studied using the K-S method. To compare the groups, the ANOVA method was used and if there was a significant difference between the groups, the Tuckey test was used. The results of each group were calculated and reported as mean and standard error (Mean ± SE). The value of $P<0.05$ was considered a significant difference.

3. RESULTS

3.1. Biochemical results

Biochemical results showed that alcoholic extract in 100, 200 and 400 mg/kg of VOE reduced serum glucose levels compared to the STZ group (Figure 1). Serum albumin and urea in the STZ group significantly were higher than the controls. There was not significant statistical differences in serum albumin, creatinine and urea between VOE treated groups and STZ group. (Figure 1).

Urinary analysis showed that a dose of 100 mg/kg VOE was not effective on urinary markers. However, the group receiving a dose of 200 mg/kg VOE all urinary parameters such as albumin, urea, and creatinine reduced compared with the STZ group ($P<0.05$). Meanwhile, 400 mg/kg dose of VOE reduced the level of urine albumin in comparison with the STZ group ($P<0.001$)

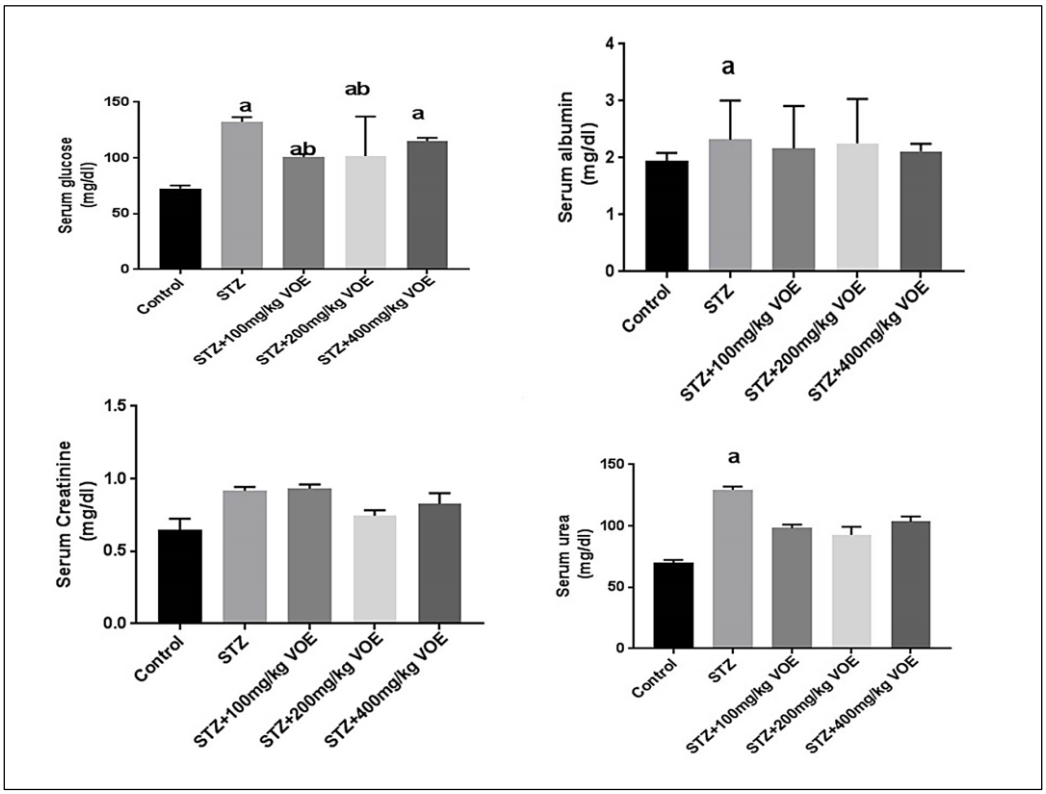

Figure 1. The effect of VOE on serum biochemistry parameters of male diabetic rats.
Values are expressed in mean ± S.E. a: significant values compared to the control group, $p<0.001$ and b: significant values compared to the STZ treated group. $p<0.05$.

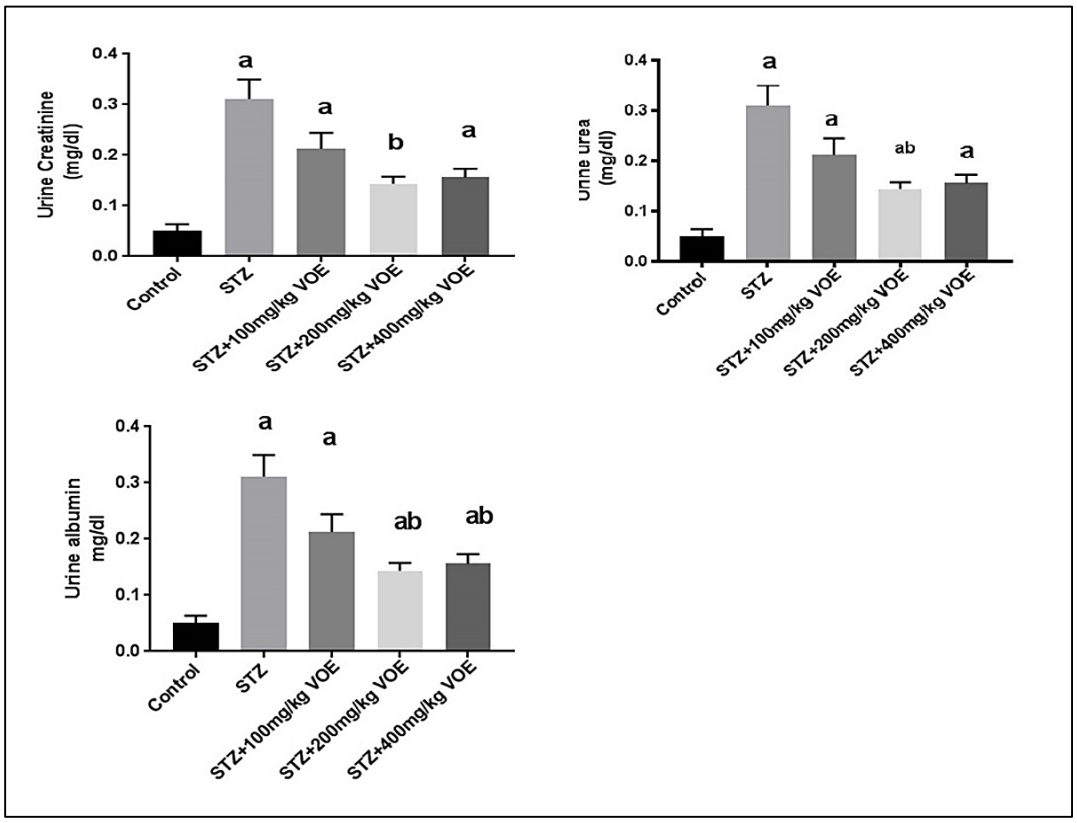

Figure 2. The effect of VOE on urinary biochemistry parameters of male diabetic rats.
Values are expressed in mean ± S.E. a: significant values compared to the control group, $p<0.001$ and b: significant values compared to the STZ treated group. $p<0.01$.

(Figure 2). Creatinine level in doses of 100 mg/kg and 400 mg/kg VOE reduced insignificantly compared with the STZ group (Figure 2).

3.2. Malondialdehyde (MDA) Assay

Serum MDA level in the STZ group increased about 3 folds in compare with controls ($p<0.001$). Treatment with 100 mg/kg and 200 mg/kg VOE significantly reduced serum MDA level compared with STZ treated group. VOE in a dose of 400 mg/kg reduced MDA level, however, it was not significant (Figure 3).

3.3. Histologic results

Histologic study showed renal corpuscles expanded and the area of both tuft glomerular and renal corpuscles in the STZ group significantly increased compared to controls ($p<0.001$). Bowman space observed larger in the STZ group. The colony of inflammatory cells in the interstitial tissue and around blood vessels observed in the STZ group. Epithelial height of PCT and DCT reduced in STZ treated group compared to controls (Figure 4). The abnormalities were restored by treatment with VOA especially in the dose of (200 mg/kg). Thus, the VOE extract might repair the damaged kidney morphology caused by diabetes (Figure 4 and Table 1).

4. DISCUSSION

The present study showed the administration of STZ caused diabetes in animals. In this study, we used VOE in doses of 100mg/kg, 200 mg/kg and 400 mg/kg to determine its anti-diabetic and renoprotective effects. Also, 200 mg/kg VOE reduced the urinary amount of albumin, urea, and creatinine compared to the STZ group. STZ increased the area of both glomerular tuft and renal corpuscles but VOE in different doses reduced them. Medicinal plants have used for the treatment of diabetes since 1550 B.C. Various herbs, spices, and plant materials have been used for this purpose throughout the world[13]. A decrease in serum albumin, urea and creatinine levels following some doses of VOE may be due to inhibition of oxidative phosphorylation process, which resulted in a reduction of protein absorption, protein synthesis, and an elevation in the catabolic process[16]. Maybe an elevation of glucose concentration in serum or tissues resulted in the elevation of ROS formation

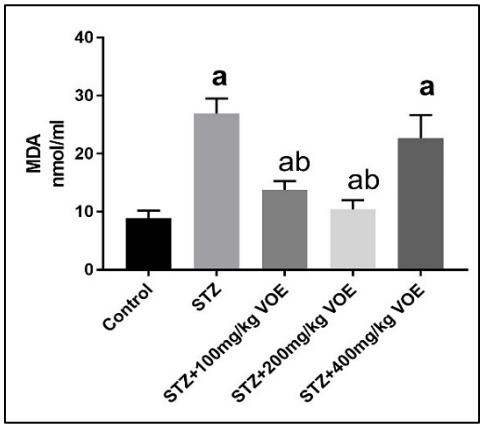

Figure 3. Serum MDA levels in groups. a: significant data compared with controls $p<0.001$, b: significant data compared with the STZ treated group $p<0.001$. Data are presented in mean± S.E.

Table 1. The effect of the VOE on the morphometric parameters of kidney tissue in diabetic male rats.

Groups	Control	STZ	STZ+100 mg/kg VOE	STZ+200 mg/kg VOE	STZ+400 mg/kg VOE
Glomerular tuft area (µm^2)	5572±610	14440±02^a	9724±410^b	7438±341^b	8971±431
Renal corpuscle area (µm^2)	8918±419	22283±70^a	12321±640^b	10456±651^b	12368±410^b
DCT epithelial height (µm)	9.28±1.6	5.74±0.9^a	8.34±1.5^b	8.78±1.7^b	7.38±1.5
PCT epithelial height (µm)	17.78	10.38	13.24^b	13.65^b	12.52

Values are expressed in mean ± S.E. a: Significant values compared to the control group, p <0.001, b: significant values compared to the STZ treated group. p <0.05. PCT: proximal convoluted tubules. DCT: Distal convoluted tubules

and therefore it may have a role in the pathology of diabetes nephropathy[17].

Oxidative stress is one of the most important causes of diabetes. Diabetes trough some mechanisms such as hyperglycemia, hyperlipidemia, and hypertension induces oxidative stress[18]. Therefore, it seems antioxidant therapy may protect or prevent diabetes complications. Our study confirmed that VOE reduced serum MDA levels as a marker of oxidative stress in comparison with the STZ group. In this regard, it has been shown that the water extract of VOE has free radical scavenging activity[19]. Similarly, *Benafsha Atri* (a kind of *Viola*), reduces the level of serum glucose which is in confirmation with our study. A previous study has been shown that the *viola* contains anthocyanin[20]. Anthocyanins have anti-bacterial, antihistamine, anti-allergic, anti-malarial, lipid, cholesterol, and anti-diabetic effects[21]. Also, the presence of liver protective flavonoids has been proven in this plant[21]. Similarly, phytochemicals of berries have potential health activities against T2DM[6].

Researches have shown VOE contains a combination of alkaloids, glycosides, tannins, steroids, triploids, saponins, flavonoids, methyl salicylates, mucilages and vitamins C[19-22]. About 30 cyclotides are known in both root and air organs of the *V. odorata* plant[23]. The root of this plant contains a violin alkaloid that is used as an expectorant[9]. Various reports have shown that the anti-diabetic properties of medicinal plants are due to the presence of saponin. The ability of saponin to reduce plasma glucose levels has made saponins an excellent candidate for diabetes mellitus[24]. So far, flavonoid glycosides, mainly derivatives of kaempferol, quercetin and apigenin have been reported from *V.odorata*[20].Flavonoids through improvement of lipid profile, antioxidants level, and glycemic level have therapeutic effects on diabetes. Among them, rutin, as a flavonoid which is reported in *V.odorata*, indicated significant biological properties like antioxidant, nephron-protective, neuroprtective and anti-inflammatory[25].

Previous studies have shown the effects of perfume violet powder on liver and kidney function following tetrachloride (CCL4) liver toxicity[10]. The exact mechanisms of the effect of VOE on renal tissue is unknown and needs more investigations.

5. CONCLUSIONS

In conclusion, the results of the present study showed that VOE has hypoglycemic properties VOE may protect renal function induced by STZ, especially in a dose of 200 mg/kg and

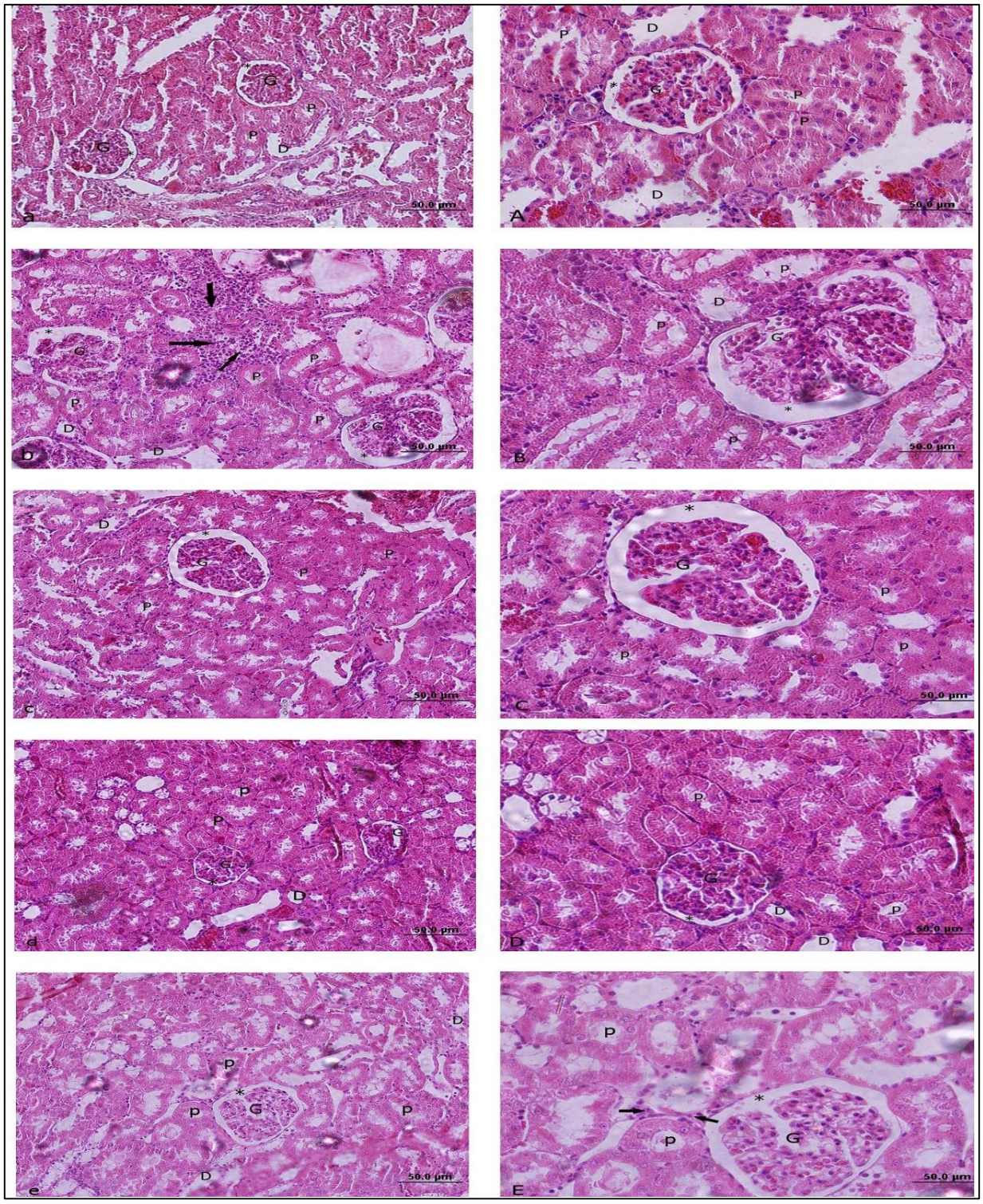

Figure 4. Photomicrograph of the rat kidney tissue. a and A: Control group, b, and B: STZ group, c, and C: STZ+ 100 mg/kg VOE group, d and D: STZ + 200 mg/kg VOE, e and E: STZ + 400 mg/kg VOE. G: Glomerulus tuft, P: proximal convoluted tubule, D: distal convoluted tubule, * shows bowman space, arrows represent the aggregation of inflammatory cells. In figure b and B: STZ treated group please note to an expansion of renal glomerulus tuft, renal corpuscles, and Bowman's space. Also, note to a reduction of epithelial height in proximal and distal convoluted tubules. Note that STZ+100mg/kg VOE and STZ+200mg/kg VOE have restored most of the abovementioned structural abnormalities in the kidney. H&E staining. 200× and 400×.

through reduction of oxidative stress.

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Conflict of interest
The results of this article are not in conflict with the interests of the authors.

Ethical approval
This study was done by following the guidelines provided by the Animal Laboratory. All principles of animal work were carried out according to the ethics committee approved by Guilan University of Medical Sciences (animal protocol number: IR.GUMS.REC.1395.226).

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