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NEPHROPROTECTIVE EFFECT OF *LAVANDULA OFFICINALIS* L. ETHANOLIC EXTRACT IN ALLOXAN INDUCED DIABETIC RATS

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ABSTRACT

Diabetic nephropathy is chronic complication of diabetes that induced by hyperglycemia with increased generation of ROS, so the present study was conducted to determine the probable nephroprotective activity of *Lavandula officinalis* L. ethanolic extract in the alloxan-induced diabetic rats. Male rats were divided into four groups as following: control group (HC) received saline (0.9% i.p.); diabetic group (DC) received single i.p. administration of alloxan (120mg/kg b.w); First dose treatment group (FT) received LOE (100mg/kg b.w) i.p. for 21 consecutive days; second dose treatment group (ST) received LOE (200mg/kg b.w) i.p. for 21 days, both treatment groups received a single dose of alloxan (120mg/kg) for inducing diabetes type I. kidney tissue dissected to measure activity of some antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation (MDA) by spectrophotometer. Blood samples collected to measure urea and creatinine in the serum. This study revealed that intraperitoneal administration of LOE for 21 days, afforded significant nephroprotection against alloxan-induced elevation in serum marker Urea (38%) and Creatinine (39.45%) $P<0.05$, kidney antioxidant enzymes activities SOD (72.31%) $P<0.01$, CAT (42.53%) and kidney lipid peroxidation (45.64%) $P<0.05$ compared to DC. The present study indicated the nephroprotective effect of LOE in diabetic rats. These effects are in partly due to its potent antioxidant properties.

KEYWORDS: Alloxan, Diabetes, *Lavandula officinalis*, Nephroprotective, Oxidative stress.

1. INTRODUCTION

Diabetes mellitus (DM), a metabolic disorder is a major public health problem in both developed and developing countries. It is the fourth or fifth leading cause of death in most high-income countries. DM increases risk of several serious health problems or complications including hyperlipidemia, poor metabolic control, nephropathy, hepatopathy.[1-3] These complications are considered the leading causes for death among these patients. Thus, an early control of DM is recommended as one of the main strategies to prevent these complications and increase the life span of these patients. During the last several years. Alternative treatment for diabetes has become popular including medicinal herbs and nutritional completion.[4] Hyperglycemia leads to increased production of reactive oxygen species (ROS). These free radicals are implicated in the complications of DM like nephropathy. Plants have been the basis for medical treatments through much of human history, and such traditional medicine is still widely practiced today. Drugs which have natural origin are less toxic and considered to have low side effect than synthetic drugs.[5] *Lavandula officinalis* which is commonly known in Iran as "Ostokhoddous" is one of these plants which have traditionally been used in medicine. Some various biological activities of LOE such as anti-tumor, antimicrobial and anti- inflammatory have been shown in recent years.[6-11] However, no detailed scientific study seemed to have been carried out to assess the nephroprotective activity of LOE in diabetic subjects. This study was to investigate the probable nephroprotective effect of LOE in the alloxan-induced diabetic models.

2. MATERIALS AND METHODS

2.1. Chemicals

Ethylene diamine tetra acetic acid (EDTA) and trichloroacetic acid (TCA) were obtained from (Sigma-Aldrich Chemical Co. Ltd USA). Thiobarbituric acid (TBA) was obtained from Merck Co. (Germany).

Alloxan monohydrate was obtained from across organics, (New Jersey, USA).

2.2. Plant Materials

Lavandula officinalis (LO) were obtained from suburbs of Shiraz city (Iran) at the beginning of summer 2016. The areal part of the plant was air-dried and then ground into powder; in all the steps, the components were protected from direct sunlight. The powder was kept at 8°C.

2.3. Extraction

The air-dried LO were ground into powder, 200 g of which was mixed with an ethanol: water (5:1) solution at 25 ± 2°C. The solvent was completely removed by rotary vacuum evaporator at 50°C. Afterwards, LOE was kept in 4°C until using time.

2.4. Animals and Treatment

The animals (wistar strain male albino rats [200 - 250g]) were obtained from Pastor Institute (Tehran, Iran). Animals were kept in a room with 22 ± 2°C, temperature and light and dark cycles (12/12 hours for each), for 1 week before and during the experiments. Animals were fed with a standard diet and clean drinking water ad libitum. All animal procedure was performed according to the National Institute of Health's Guide for the care and use of laboratory animals. Twenty eight male albino wistar rats were randomly divided into four groups as following: healthy control group (HC) receive saline (0.9 % i.p.)during the experimental days; diabetic group (DC) receive single i.p. administration of alloxan (120mg/kg b.w); First dose treatment group (FT) received a single dose of alloxan (120mg/kg) and LOE (100mg/kg b.w) i.p. for 21 consecutive days and second dose treatment group (ST) receive single dose of alloxan (120mg/kg) and LOE (200mg/kg b.w) i.p. for 21 days.

2.5. Preparation of kidney homogenate

After termination of treatment days, in day 22, animals were scarified. The kidney tissues were homogenized in KCl (10 mM) phosphate buffer (1.15%) with EDETA (pH= 7.4) and centrifuged at 3000 rpm for 30 minutes. The supernatants were collected to be used for measurement of malondialdehyde (MDA), catalase (CAT) superoxide dismutase (SOD). The total protein content was determined based on Lowry's method.[12]

2.6 Designation of lipid peroxidation

Kidney homogenate lipid peroxidation was measured based on formation of Thiobarbituric acid reactive substance (TBARS). MDA forms were mixed with TBA, which was measured by spectrophotometer at 532 nm. The concentration of MDA was computed based on the absorbance coefficient of the TBA-MDA complex (ε = 1.56 × 105/M/cm), and presented as nmol/mg of protein.[13]

2.7 Antioxidant enzymes activity

For the measurement of catalase activity, one unit of catalase was required to decompose 1 µM of H2O2 in 1 minute. Decomposition level of H2O2 was determined by spectrophotometer at 240 nm for 2 minutes. The enzyme activity was presented as U/mg of protein.[11] The SOD activity was assayed as described by Winterbourne et al. One unit of enzyme activity (as amount of SOD) was determined to produce a 50% inhibition of NBT reduction and the specific enzyme activity was presented as units per milligram of total protein.

2.8 Assessment of kidney function

Blood urea (Urea) and creatinine (Creatinine) content was were measured using commercially available diagnostic kit.

2.9 Histopathological study

After the experiment, the animals were scarified and their kidney removed immediately. The kidney tissue was fixed in 10% formalin, the paraffin blocks of kidney were provided, and then histological sections of 5 - 6 µm in thickness were prepared. The samples were stained with hematoxylin-eosin solution and observed under a light microscope. 2.10 Statistical Analysis

Data were presented as Mean ± SEM. One-way analysis of variance (ANOVA) followed by multiple comparison with the Tukey post-hoc test was used to compare different parameters between the groups. The significance level was considered at $P<0.05$.

3. RESULT

3.1 The effect of LOE on Lipid peroxidation

Evaluated level of malondialdehyde content in homogenate in rat kidney is shown in Table 1. MDA contents in the kidney homogenate were significantly ($P<0.05$) increased in the diabetic group (0.675±0.078) compared to the control group. MDA level of LOE treated groups (100 and 200 mg/kg) were significantly ($P<0.05$) decreased (0.373±0.039) compared to the diabetic group.

3.2 The effect of LOE on antioxidant enzyme activity in the kidney tissue

The effect of LOE on SOD, CAT activities in kidney are shown in Table 1. Antioxidant enzymes activity in diabetic group (5.361±0.406 and 154.84±6.11) is lower than normal control. The activities of these enzymes in LOE treated groups significantly ($P<0.01$ and $p<0.05$) increased SOD (17.083± 0.665) and catalase (264.83±12.745) compared to the diabetic group.

3.3 Effect of LOE on kidney functions

The exposure of rats to alloxan led to kidney dysfunctions Urea (230.3±22.36) and Creatinine (0.73±0.065) as indicated by the blood Urea and Creatinine level (Table 2). The activities of these parameters in LOE treated group, Urea (124±36.56) and

Creatinine (0.575±0.036) was significantly P<0.05 lower than diabetic control.

3.4 The effect of LOE on histopathology of kidney

Administration of Alloxan caused glomerular and tubular injury. Treatment with LOE ameliorated the change to near normal histology (Figure 1).

Table 1: Oxidative stress parameters indicate the kidney function.

Groups	SOD (U/mg protein)	CAT (nmol H_2O_2/min/mg protein)	MDA (nmol/mg protein)
HC	13.82 ± 2.39	189.22 ± 25.11	0.42 ± 0.089
DC	4.91 ± 0.96[a]	144.34 ± 36.09[a]	0.74 ± 0.094[a]
FT	10.32 ± 1.45	259.87 ± 81.23	0.41 ± 0.050
ST	14.26 ± 1.95[c]	265.41 ± 52.22[b]	0.39 ± 0.041[b]

a: Indicates the significance P<0.05 compared with the HC group.
b: indicates the significance P<0.05 with DC group.
C: indicates the significance P<0.01 compared with the DC group.
Note that values are Mean± SEM (n=7 in each group).

Table 2: Serum biomarkers indicates the kidney function.

Groups	Urea (mg/dl)	Creatinine (mg/dl)
HC	85.82 ± 10.39	0.85 ± 0.031
DC	220.91 ± 0.96[a]	0.79 ± 0.095[a]
FT	150.41 ± 52.22	0.41 ± 0.049[b]
ST	120.32 ± 1.45 [b]	0.54 ± 0.063

a: Indicates the significance P<0.05 compared with the HC group.
b: indicates the significance P<0.05 compared with the DC group.
Note that values are Mean± SEM (n=7 in each group).

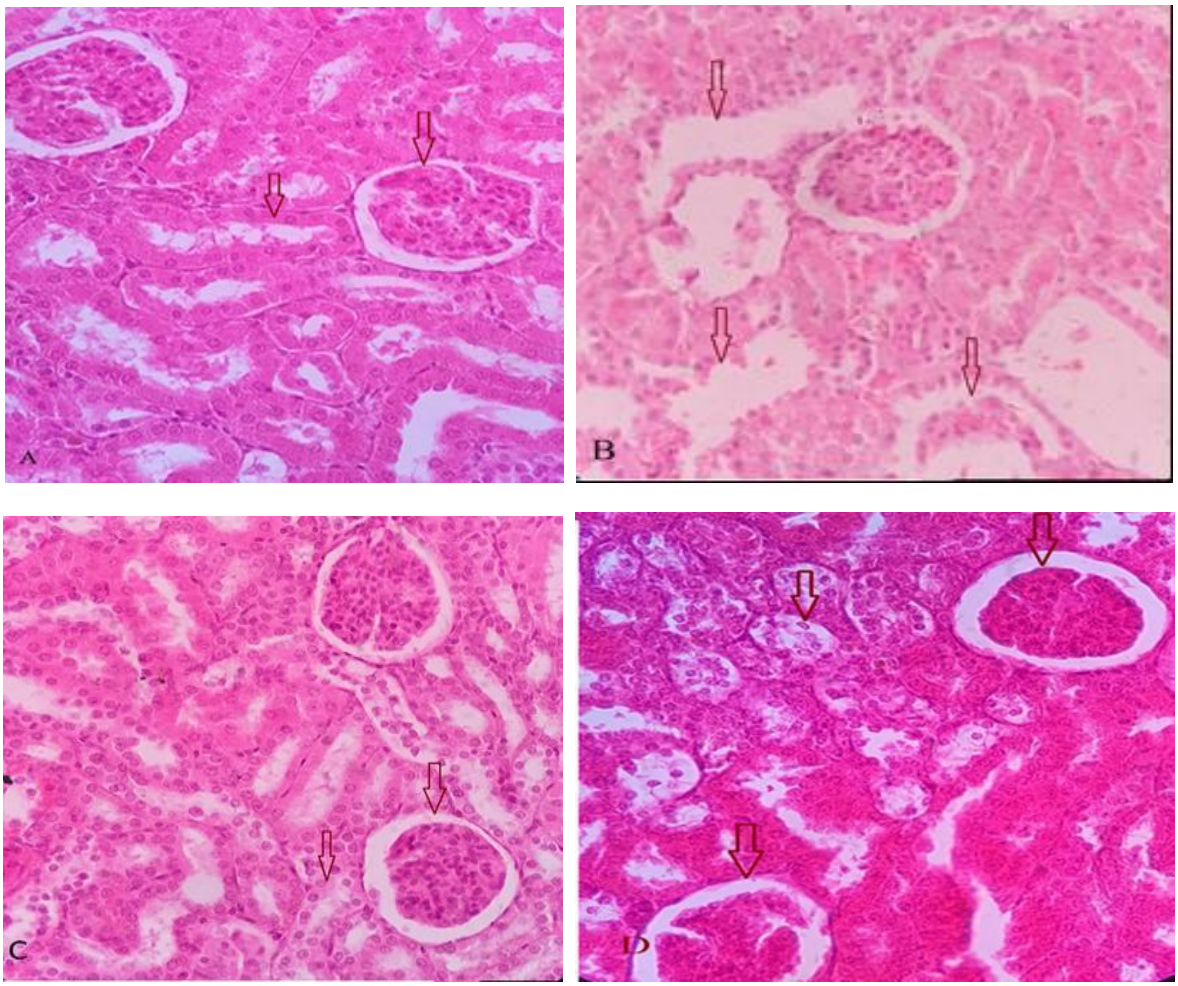

Figure 1: H and E stain: (A) Representative section from the control group. (B) Diabetic group. (C) LOE (100 ml/kg) group. (D) LOE (200 mg/kg). LOE, Lavandula officinalis extract; DBC, damaged Bowman capsule; TD, tubular degeneration; LBB, loss of border brush; PC, proximal tubule; DT, distal tubule; NGB, normal glomerulus and Bowman capsule; BB, border brush; FA, foamy appearance.

4. DISCUSSION

Our study showed the nephroprotective effect of LOE against kidney injury in diabetic rats. The major effect of pathogenesis of this disease is increased generation of ROS. In this study alloxan by its oxidative stress function cause damage in various organs like renal tissue.

Oxidative stress starts lipid peroxidation of cell membrane polyunsaturated fatty acids.[15] Lipid peroxidation shows on of the most frequent reaction resulting from the free radical attacks to biological structures as well as accumulation of oxidized lipids in the cell membrane.[16] Our results showed the reduction effect of LOE on TBARS production. In this study, alloxan administration significantly ($P<0.05$) increased the kidney MDA content, probably revealing the increase of lipid peroxidation. The significant decrease in the kidney malondialdehyde content, as a marker of lipid peroxidation, confirmed that treatment with LOE could have a great protective effect against alloxan- induced kidney lipid peroxidation.

Intracellular ROS concentration is a conclusion of their production and elimination by diverse antioxidants. Major components of the antioxidant system in mammalian cells include SOD and CAT. These enzymes have important roles in eliminating superoxide anion and H2O2 in cells.[17] SOD, an important antioxidant enzyme, catalyzes the highly reactive toxic superoxide radicals to H2O2,[18] and H2O2 decomposition to oxygen and water are catalyzed by catalase.[19,20] Our results indicated that the levels of antioxidant enzymes such as SOD and CAT decreased in the Alloxan-treated group, were recovered by LOE treatment. The protective effects of LOE in maintaining the above enzymes close to the control level increased the capacity of endogenous antioxidant defense as well as their steady state. These effects can also enhance the enzymes synthesis rates, conferring enhanced protection against oxidative stress. The histopathological evaluations of rat kidneys treated with LOE, following receiving Alloxan, showed prophylactic effect of LOE on nearly normal structures of kidney. This protection mechanism provides resistance to kidney against alloxan-induced damages, through nephron regeneration stimulation and kidney lipid peroxidation inhibition.[21] The increases of plasma Urea and creatinine activities indicated that diabetes may induce nephron dysfunction as supported by previously findings showing a necrotic kidney.[22] Therefore, the increase of Urea content in plasma may be mainly due to the nephron dysfunction.[23] On the other hand, treatment of the alloxan-diabetic rats with LOE restored the urea level. These results are in line with those obtained by Ohaeri[24] and illustrate the nephroprotective effects of LOE against alloxan- induced toxicity.

Antioxidant components of LOE may cause membrane stabilization and reverse the normalization of fluctuated biochemical profiles induced by alloxan exposure. Therefore, plant extract compounds affect the kidney by maintaining its normal function and decreasing the derangements of cell membrane. Purification of LOE active components for determining their exact protective effects on nephrons is recommended for further studies. The present study indicated the nephroprotective effect of LOE in diabetic rats.

ABBREVIATION

DM: Diabetes mellitus, HC: Healthy control, DC: Diabetic control, FT: First dose treatment, ST: Second dose treatment, LO: Lavandula officinalis, LOE: Lavandula officinalis ethanol extract, SOD: Superoxide dismutase, CAT: Catalase, MDA: Malondialdehyde.

DECLARATION OF INTEREST

The authors declared no potential conflicts of interest.

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