Protective Effect of *Salvia Leriifolia* Benth. Root Aqueous and Ethanolic Extracts on Renal Ischemia-Reperfusion in Uninephrectomized Wistar Male Rats

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Summary

The effect of *Salvia leriifolia* Benth. root aqueous and ethanolic extracts on oxidative stress, renal dysfunction was evaluated during the renal ischemia-reperfusion in uninephrectomized rats. The left kidney was exposed to warm ischemia for 60 min followed by reperfusion for 90 min. The aqueous and ethanolic extracts of *S. leriifolia* (0.1, 0. 2 and 0.4 g/kg) were injected intraperitoneally prior to induction of ischemia. Normal saline was injected to control group and a sham group that did not have ischemia-reperfusion. The aqueous and ethanolic extracts pretreatment resulted in a significant reduction in the free radical-mediated lipid peroxidation as indicated by a decrease in the MDA levels, at various dose levels (except 0.1 mg/kg for aqueous extract). The aqueous (0.2 and 0.4 mg/kg) and ethanolic (0.4 mg/kg) extracts pretreatment increased antioxidant power (FRAP value) of kidney homogenate samples. Both extracts pretreatment caused a significant and dose dependently elevation in total thiol concentration, as compared with control group. This study showed that the aqueous and ethanolic extracts of *S. leriifolia* root may be useful for the prevention of renal ischemia-reperfusion-induced oxidative injury in rats. These effects may have been mediated, at least partially, via antioxidant capacity.

Key words: *Salvia leriifolia* Benth. Lipid peroxidation, Protein oxidation, Ischemia-reperfusion kidney injury, Renal failure.

INTRODUCTION

Salvia is an important genus consisting of about 900 species in the family Lamiaceae which has different pharmacological activities [1]. *Salvia leriifolia* Benth. (vernacular names such as Nuruozak and Jobleh) is a perennial herbaceous plant that grows in south and tropical regions of Khorasan and Semnan provinces, I. R. Iran. The different pharmacological activities of this plant such as the attenuation of morphine dependence [2], hypoglycemic [3], analgesic and anti-inflammatory [4-5], anticonvulsant [6], antiulcer effects[7], anti-anxiety [8] and antibacterial activities [9] were evaluated in mice or rats. Recently pharmacological effects of this plant were published in a review.[10]

Using thin-layer chromatography (TLC), it has been shown that different fractions isolated from *S. leriifolia* leaves have appreciable antioxidant effect. Whole methanolic extract, precipitates of methanolic extract and most separated fractions showed more antioxidative activity than α–tocopherol [11]. Our previous studies also showed that different part of *S. leriifolia* such as root, leaves and seed have neuroprotective activities against lipid peroxidation in cerebral ischemia [12-13] and recently we showed that *S. leriifolia* root extracts have some protective effects on different markers of oxidative damage in muscle tissue injury caused by lower limb ischemia-reperfusion [14]. Thus, in this study, we decided to evaluate the protective effect of this plant on ischemic injured oxidative stress in kidney tissue of rats.

MATERIALS AND METHODS

Animals

Adult male Wistar rats weighting 200-300 g were used throughout the study. All of them were kept in the same room under a constant temperature (22 ± 2 °C) and illuminated with food pellets and water available *ad libitum*. The light system was 12-12h. The animals were housed in plastic cages and went through a 12 h before experiments to that place. All animal experiments were carried out in accordance with Mashhad University of Medical Sciences, Ethical Committee Acts.

They were anaesthetized with 60 mg/kg ketamine and 10 mg/kg xylazine hydrochlorides given intraperitoneally (i.p.). Animals were divided into eight groups, each of which contained 6-8 rats. Group 1 was the sham group in which only surgery was done, group 2 was the control group in which saline solution was given intraperitoneally (i.p.), groups 3-8 were given three concentration of macerated aqueous and ethanolic extracts (0.1, 0.2 and 0.4 g/kg) i.p. prior to induction of ischemia.

Preparation of aqueous and ethanolic *S. leriifolia* Benth. Extracts

S. leriifolia Benth. was collected from Bajestan (Khorasan province, Northeast of Iran).
In the maceration method, 100 g of plant powder were macerated in 1000 ml ethanol for three days and for preparation of decoction extract, 100g of plant powder was boiled for 15 min. in 1000 ml distilled water. The mixture was subsequently filtered and concentrated under reduced pressure at 35ºC [12].

Chemicals

DTNB (2, 2´-dinitro-5, 5´-dithiodibenzoic acid), TPTZ (2, 4, 6-tri (2-pyridyl)-1, 3, 5-triazine), TBA (2-thiobarbituric acid), n-butanol, tris, Na_2EDTA, sodium acetate, glacial acetic acid, phosphoric acid, potassium chloride, tetramethoxypropane (TMP), ferric chloride ($FeCl_3.6H_2O$), ferrous sulfate and hydrochloric acid was obtained from Merck. [15].

Induction of renal ischemia-reperfusion injury (IRI)

The animals were subjected to left renal warm ischemia for 60 minutes, and reperfusion for 90 minutes. under intraperitoneal ketamine/xylazine anesthesia (60 mg/kg and 10 mg/kg) and through a midline incision, the abdominal contents were displaced to the right side. The left renal artery and vein were dissected and the perirenal fat was preserved. At the end of the ischemic period, the abdominal cavity was reentered, the ligature was removed and reperfusion was supplied. At the 90th minutes of reperfusion left kidney was removed and was used for biochemical assays [16-17]

The kidney tissue was homogenized in cold KCl solution (1.5%) to give a hemogen suspension (10%). It was used for three tests [18].

Tiobarbituric acid reactive species (TBARS) measurement

The test was done for the determination of lipid peroxidation. Thiobarbituric acid reactive products (TBAR) of lipid peroxidation were determined in the kidney tissue (Just like malondialdehyde, MDA) [17]. MDA is final product of lipid peroxidation and its complex with TBA, is red and has maximum absorbance at 532 nm. 0.5 ml of suspension (that was explained before) was poured to a tube with 10 ml capacity and 3 ml phosphoric acid (1%) and 1 ml TBA (Thiobarbituric acid) add it and then tube was put in boiling water bath for 45 minuets. After this time, suspension was cooled and 4 ml n-butanol was poured it and was put in vortex for complex mixing (1 minute), then with centrifuge (20000 rpm speed, 20 minutes) the red-colored upper phase was separated and obtained. The standard curve with MDA (0-40 μM) was prepared [15, 19].

Total SH (sulfhydryl) groups assay

The test was for tiol groups. 100 μL of the first hemogenized suspension was taken and 2 ml Tris buffer was added it, then 40 μL of the Elman reagent was added and absorbance at 413 nm was measured against a reagent blank. A millimolar absorption coefficient of 13.6 was used for calculation of –SH group content. Total thiol concentration (mM) = $(A_2\text{-}A_1\text{-}B) \times 1.07/0.05 \times 13.6$ [20].

Ferric Reducing / Antioxidant Power (FRAP) assay

The test was for changing Fe^{3+}, in FRAP reagent, to Fe2+ (reduction reaction). 50 μL of kidney homogenate mixture add to 1.5 ml FRAP reagent (25 ml Phosphate Buffer, 2.5 ml Ferric chloric and 2.5 ml TPTZ solution that 5 minutes were put in 37°C water bath, Benmarry) and then this suspension again 15 minutes were heated in water bath (37 °C). After this time, absorbance of the solution at 293 nm was read in spectrophotometer.

Data of lipid peroxidation was reported as MDA, nanomol per each gram of tissue and protein oxidation as micromole/g tissue for FRAP test and milimolar for DNTB test [21].

Statistical analysis

Data are expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey-Kramer *post-hoc* test for multiple comparisons. The p-values less than 0.05 were considered to be statistically significant.

RESULTS

Change of MDA levels by extracts

There was an increase (54% in aqueous and 30% in ethanolic extracts) in the MDA levels following IRI as compared with sham-operated animals (42.9 ± 4.3 vs. 23.4 ± 2.8 nmol/g tissue, $p<0.01$, Fig. 1, and 8.3 ± 1.3 vs. 2.5 ± 0.34 nmol/g tissue, $p<0.001$, Fig. 2). In *S. leriifolia* root aqueous extract -pretreated groups, a reduction in TBARS levels was observed dose dependently. At all doses, the ethanolic extract also reduced lipid peroxidation products in dose dependent manner (Fig. 1 and 2).

Change of FRAP value by extracts

Ischemia-reperfusion caused a significant reduction in FRAP value (49 % in aqueous and 55% in ethanolic extract) of kidney homogenate samples as compared with sham-operated animals (5.2 ± 0.6 vs. 2.6 ± 0.31 µmol /g tissue, p<0.001 and 1.5 ± 0.07 vs. 0.8 ± 0.06 µmol /g tissue, p<0.001). The aqueous extract pretreatment increased antioxidant power (FRAP value) of kidney homogenate samples, dose dependently (Fig 3.). Only at high dose, the ethanolic extract increased antioxidant power (Fig 4).

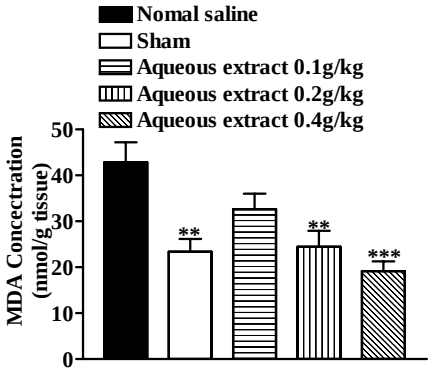

Figure 1: Effect of aqueous *Salvia leriifolia* Benth. root extract on lipid peroxidation following renal ischemia-reperfusion injury. MDA levels were measured in 10% homogenates of kidney samples from rats subjected to 60 minutes of ischemia and 90 minutes of reperfusion. All drugs were administrated intraperitoneally prior to induction of ischemia. Values are mean ± SEM (n=6). **P<0.01, ***P<0.001 as compared with vehicle (normal saline) treated animals (One-way ANOVA following by Tukey-Kramer test).

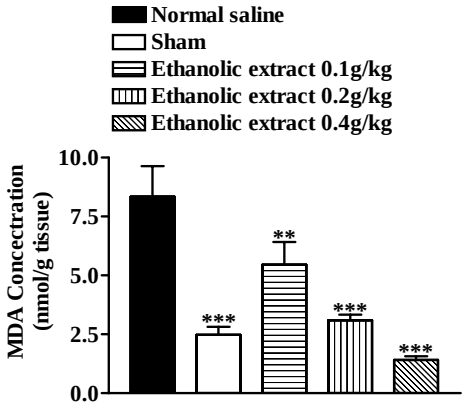

Figure 2: Effect of ethanolic *Salvia leriifolia* Benth. root extract on lipid peroxidation following renal ischemia-reperfusion injury. MDA levels were measured in 10% homogenates of kidney samples from rats subjected to 60 minutes of ischemia and 90 minutes of reperfusion. All drugs were administrated intraperitoneally prior to induction of ischemia. Values are mean ± SEM (n=6). **P<0.01, ***P<0.001 as compared with vehicle (normal saline) treated animals (One-way ANOVA following by Tukey-Kramer test).

Effect of extracts on total thiol concentration

Following ischemia-reperfusion injury a significant reduction (48 % in aqueous and 34% in ethanolic extract) in total SH groups (0.61 ± 0.03 vs. 0.29 ± 0.03 mM, p<0.001 and 0.59 ± 0.03 vs. 0.2 ± 0.03 mM, p<0.001) in kidney homogenate samples were observed. Both extract pretreatment caused a significant and dose dependently elevation in total thiol concentration, as compared with control group (Figs. 5 and 6).

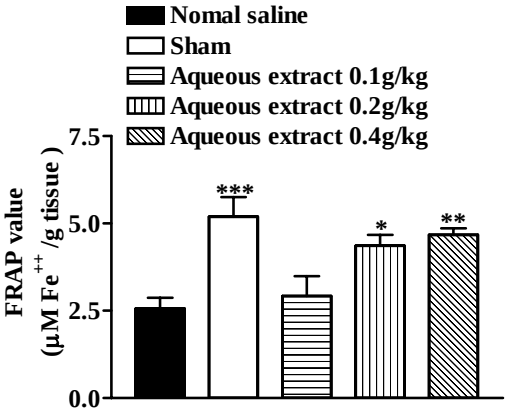

Figure 3: Effect of aqueous *Salvia leriifolia* Benth. root extract on lipid peroxidation following renal ischemia-reperfusion injury. FRAP values were measured in 10% homogenates of kidney samples from rats subjected to 60 minutes of ischemia and 90 minutes of reperfusion. All drugs were administrated intraperitoneally prior to induction of ischemia. Values are mean ± SEM (n=6). *P<0.05, **P<0.01, ***P<0.001 as compared with vehicle (normal saline) treated animals (One-way ANOVA following by Tukey-Kramer test).

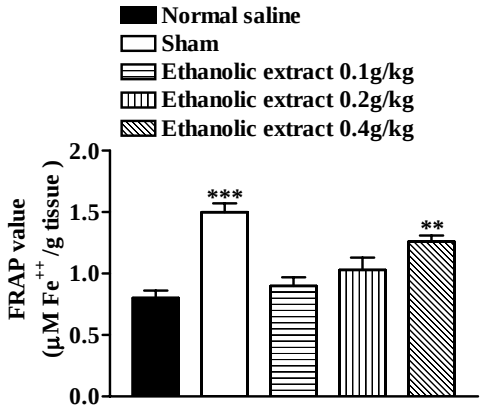

Figure 4: Effect of ethanolic *Salvia leriifolia* Benth. root extract on lipid peroxidation following renal ischemia-reperfusion injury. FRAP values were measured in 10% homogenates of kidney samples from rats subjected to 60 minutes of ischemia and 90 minutes of reperfusion. All drugs were administrated intraperitoneally prior to induction of ischemia. Values are mean ± SEM (n=6). **P<0.01, ***P<0.001 as compared with vehicle (normal saline) treated animals (One-way ANOVA following by Tukey-Kramer test).

DISCUSSION

In this study *S. leriifolia* Benth. root aqueous and ethanolic extracts changed the biochemical markers of oxidative reactions in order to protect kidney against ischemia reperfusion in rats.

An important role of reactive oxygen species (ROS) is their ability to cause oxidative damages to lipids, proteins and nucleic acid [22-24). Free radical-induced lipid peroxidation produces cytotoxic aldehydes, including malondialdehyde (MDA), 4-hydroxynonenal (HNE) and acrolein [25].

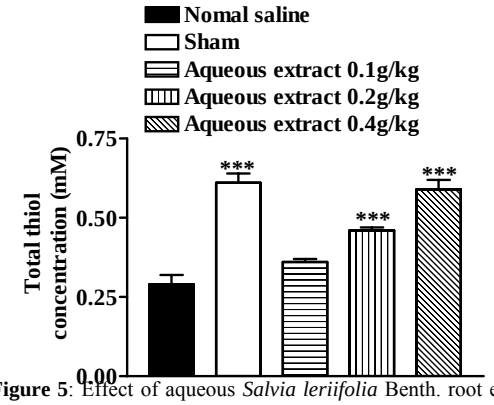

Figure 5: Effect of aqueous *Salvia leriifolia* Benth. root extract on lipid peroxidation following renal ischemia-reperfusion injury. Total sulfhydryle (SH) group were measured in 10% homogenates of kidney samples from rats subjected to 60 minutes of ischemia and 90 minutes of reperfusion. All drugs were administrated intraperitoneally prior to induction of ischemia. Values are mean ± SEM (n=6). *P<0.05, **P<0.01, ***P<0.001 as compared with vehicle (normal saline) treated animals (One-way ANOVA following by Tukey-Kramer test).

Calapai et al and lazzarino ea al showed that release of MDA occurs during brain ischemia followed by reperfusion [26-27]. Several studies have shown that the antioxidant compounds and free radical scavengers inhibit lipid peroxidation caused by free radicals (26, 28-29].

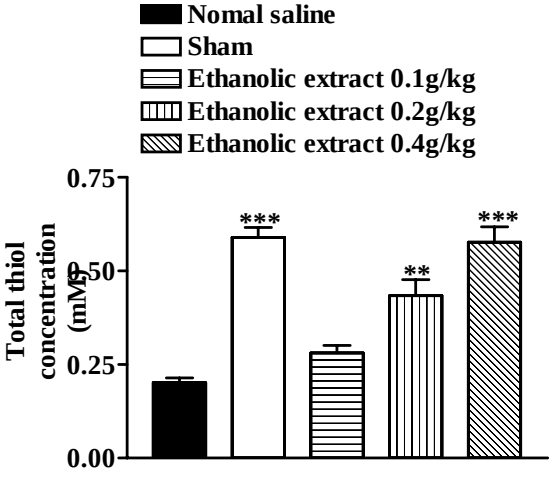

Figure 6: Effect of Ethanolic (Ethanolic) *Salvia leriifolia* Benth. root extract on lipid peroxidation following renal ischemia-reperfusion injury. Total sulfhydryle (SH) group were measured in 10% homogenates of kidney samples from rats subjected to 60 minutes of ischemia and 90 minutes of reperfusion. All drugs were administrated intraperitoneally prior to induction of ischemia. Values are mean ± SEM (n=6). *P<0.05, **P<0.01, ***P<0.001 as compared with vehicle (normal saline) treated animals (One-way ANOVA following by Tukey-Kramer test).

Present study showed that *Salvia* root extracts suppressed the increase of MDA levels in rat kidney and therefore inhibited lipid peroxidation following ischemia-reperfusion injury. Inhibition of lipid peroxidation and anti-ischemic effects is probably related to the antioxidant properties and free radical scavengering of the *Salvia* extracts. It has been shown that different fractions isolated from *S. leriifolia* leaf have appreciable antioxidant effect.

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