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Persian Sage *(Salvia Rhytidia)* Essential Oil can Ameliorate the Renal Ischemia-Reperfusion Injuries in Rat

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Abstract

Objective- In the present study the role of essential oil of *Salvia rhtidia* (SR), in ameliorating renal ischemia reperfusion (IR) injuries is evaluated.
Design- Experimental in vivo study.
Animals- 48 male healthy Wistar rats between 250-300 grams.
Procedures- The animals were randomly divided into eight groups of six rats. In ischemic groups (groups A-D), under general anesthesia both renal pedicles were approach from ventral midline and occluded for 40 min by Rumel tourniquet. Groups A and B received SR essential oil 48, 24 and 1 h before operation, whereas group C and D received the same volume of normal saline (NS). In Sham groups (E-H) all the procedures were like groups A-D, except occluding the renal pedicles. The period of reperfusion in groups A, C, E, and G was 1hour, whereas in groups B, D, F, and H was 24 hours, when blood samples were taken to evaluate blood urea nitrogen (BUN) and creatinine followed by sacrificing the animals for histopathological and ultrastructural studies.
Results- IR resulted in increase in BUN and Creatinine significantly. The most increase was in group D (40min ischemia, 24h reperfusion with NS). Histopathogically and ultramicroscopically the most prominent changes including severe acute tubular necrosis

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(ATN), hemorrhage and thiroidization, cytoplasmic vacuolization, swollen mitochondria with reduction in the number of cristae, pyknotic nuclei with abnormal chromatin condensation were also in group D.
Conclusion and Clinical Relevance- This study showed that SR essential oil can protect the kindly from IR injuries, due to antioxidative effects of the drug.
Key Words- Ischemia Reperfusion Injury, Kidney, *Salvia Rhytidia*, Rat.

Introduction

Ischemia-reperfusion (IR) injury is a well-documented phenomenon, the nature of which still is not very clear despite a vast body of research on the subject [1] Tissue subjected to a period of ischemia undergoes morphological and functional damage, which increases during the reperfusion phase.[2] Endothelial cell injury, leukocyte adhesion, platelet aggregation, release of oxygen radicals from the endothelium or leukocytes and mast cell degranulation after reperfusion are considered to be closely related and interplay in the process of microvasculatory injury induced by IR. [3] The concept of reperfusion injury has been a subject of debate for the past three decades, in which some investigators believe that all injury develops during the ischemic period whereas others argue that blood reflow extends tissue injury due to the release of oxygen-derived free radicals, dysregulation of intracellular and mitochondrial calcium, microvascular dysfunction leading to incomplete return of blood flow to areas of the microcirculation (the no-reflow phenomenon), an overzealous inflammatory reaction involving influx of various populations of immune cells, and delayed cell death due to apoptosis.[4] The phenomenon occurs in a wide range of situations including trauma, vascular reflow after contraction, percutaneous transluminal coronary angioplasty, thrombolysis treatment, organ transplantation, and hypovolemic shock with resuscitation. IR exerts multiple insults in microcirculation, frequently accompanied by endothelial cell injury, enhanced adhesion of leukocytes, macromolecular efflux, production of oxygen free radicals and mast cell degranulation [3,5] The complex, interrelated sequence of events that underlies IR injury involves priming the endothelium during ischemia to produce both free radicals and chemoattractants which, upon reperfusion, sequester and activate neutrophils, thus amplifying the injury.[2]
During IR, tissues are subjected to the destructive proinflammatory cytokines and reactive oxygen species released by inflammatory cells, leading to inflammatory injury and cell apoptosis. IR in one organ also affects the secondary organs, including liver, heart [6], kidney [7], and lung [8], and even causes multiple organ failure (MOF), a common cause of mortality. [9]
Herb medicines had traditionally played a major role in the management of human health and are still playing an active role in the health care in many countries. It has been suggested that for some herbs it is the natural antioxidants they contain conferred their biological activities.[10]
Since during the IR injuries reactive oxygen species (ROS) play the main role, using the herbs with antioxidative properties can be helpful in ameliorating the injuries. Salvia spp. has long been used in Asian countries for clinical treatment of various microcirculatory disturbance-related diseases. This herbal drug contains many active water-soluble compounds which have an ability to scavenge peroxides and are able to inhibit the expression of adhesion molecules in vascular endothelium and leukocytes.[3]
In the present study the essential oil of *Salvia rhytidea Benth*, an endemic plant from south eastern of Iran, was used to prevent IR injuries induced in kidneys of rats.

Materials and Methods

Animals

The study was performed on 48 male Wistar rats between 250-300 grams. All animals were housed in an air-conditioned room (12 h light/dark cycles with a temperature of 21°C and relative humidity of 50%). The animals were fed commercial rodent pellet food and tap water, *ad libitum.* All the procedures were conducted in accordance with the European community guidelines for laboratory animals.

Experimental groups and surgical procedures

The animals were randomly assigned into 8 groups (n=6 rats in each group):
Group A: administration of SR, 40 min ischemia, 1 h reperfusion.
Group B: administration of SR, 40 min ischemia, 24 h reperfusion.
Group C: administration of NS, 40 min ischemia, 1 h reperfusion.
Group D: administration of NS, 40 min ischemia, 24 h reperfusion.
Group E: Sham 1: administration of SR, manipulation of kidneys, sacrificed after 1h.
Group F: Sham 2: administration of SR, manipulation of kidneys, sacrificed after 24h.
Group G: Sham 3: administration of NS, manipulation of the kidneys, sacrificed after 1h.
Group H: Sham 4: administration of NS, manipulation of the kidneys, sacrificed after 24h.

The operation was performed under general anesthesia (60 mg/kg ketamine + 10 mg/kg xylazine IP). Following surgical preparations, both kidneys were approached from ventral midline and the renal pedicles were occluded by a Rumel tourniquet for 40 min after which the tourniquets were released and in groups A and C, the abdomen was temporarily closed for 1 hour, followed by sacrificing the animal. In groups B and D the abdomen was closed in two layers and the animals were transferred to their cages and sacrificed after 24 hours. In sham groups the kidneys were approached and manipulated like other groups but no occlusion of the renal pedicles performed.

Preparation and administration of SR essential oil

SR essential oil was taken from fresh aerial parts of the plants collected on mid-July,2010. The plant was positively identified by a botanist in the Department of Biology, Faculty of Science, Shahid Bahonar University of Kerman. To prepare the essential oil, 250 kg of fresh aerial parts of the plant were hydrodistilled for 3.5 h using a Clevenger-type apparatus to produce the pale yellow oil in a 0.02% w/w yield. The process was done in the Department of Chemistry, Faculty of Science, Shahid Bahonar University of Kerman.
In groups A, B, E and F, 0.2 ml/kg of 0.02% SR essential oil was administered orally, 48, 24 h and 1 h before operation. In groups C, D, G and H, the same volume of normal saline was administered.

Sampling

Before sacrificing the animals by direct intracardiac injection of 10 mg/kg thiopental sodium, blood was collected from heart, to evaluate serum BUN and creatinine.

Left kidneys were bisected along the long axis, one half kept in buffered formalin, and following routine tissue processing, 5 µ sections were made and stained with hematoxylin and eosin and studied under light microscopy. Portions from the cortex of the other halves were fixed in 2.5% glutaraldehyde and 2.5% formaldehyde in 0.1 M phosphate buffered saline (PBS) at pH= 7.2 for 4 h, and postfixed in 2% OsO4 in 0.1 M PBS (PH= 7.2) for 1 h and processed routinely for transmission electron microscopy (TEM).

Statistical Analysis

All statistical analyses were carried out using SPSS statistical software (SPSS for Windows; Chicago, IL). The one way ANOVA analysis of variance and post hoc Tukey HSD test were performed on the data of the biochemical variables to examine differences between the groups. A $p < 0.05$ was considered significant.

Results

Histopathological Observations

In group A, mild epithelial changes could be seen (Fig.1). In group B, perirenal and pericapsular hemorrhage and mild acute tubular necrosis (ATN) of the cortex were obvious (Fig. 2). In group C, very severe hyperemia and hemorrhage, glomerolopathy, and hydropic degeneration were seen (Fig. 3). In group D, severe ATN, severe hyaline cast formation (thyroidization), severe hemorrhage, very thickened capsule due to infiltration of inflammatory cells, some glomrolar changes, which occurred in all parts of the kidney could be seen (Fig. 4). In sham groups (groups E, F, G, H), the structure of the kidneys were near normal (Fig. 5).

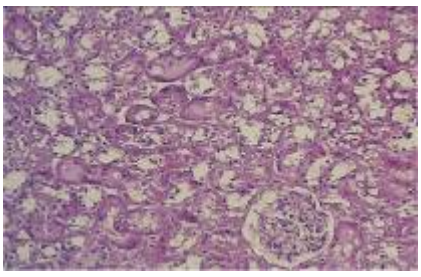

Figure 1. Mild epithelial changes in group A. H&E, ×40.

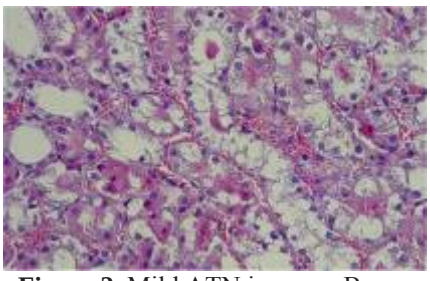

Figure 2. Mild ATN in group B. H&E, ×100.

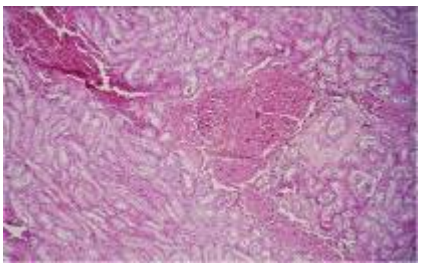

Figure 3a. Severe hemorrhage in the kidney in group C. H&E ×100.

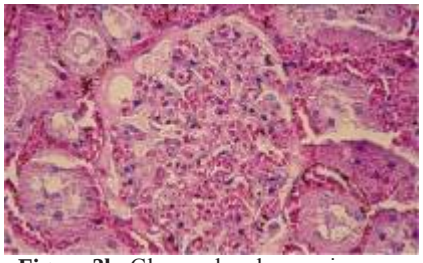

Figure 3b. Glumerolar changes in group C. H&E, ×400.

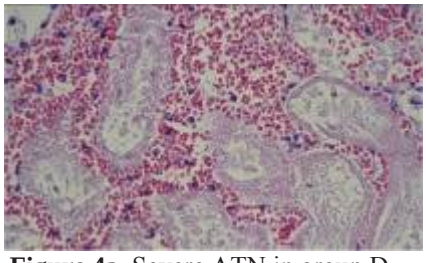

Figure 4a. Severe ATN in group D. H&E, ×400.

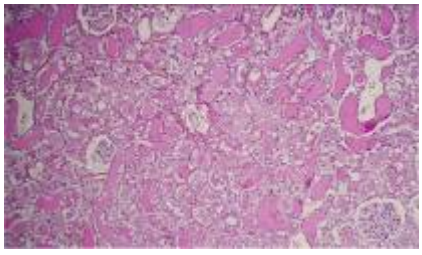

Figure 4b. Formation of hyaline cast in renal tubules (thyroidization) in group D. H&E, ×400.

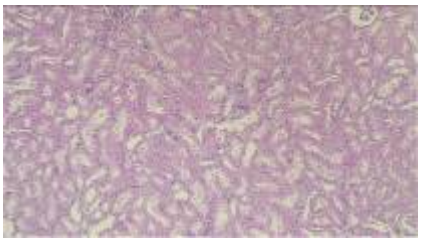

Figure 5. Normal kidney structure in seen in sham groups. H&E, ×40.

TEM Observations

The transmission electron micrograghs of the control groups (C and D) revealed severe renal tissue damage after IR , particularly in the proximal tubules. The normal tubular structure was disrupted with shedding of microvilli (brush border) into the tubular lumen, Cytoplasmic vacuolization, swollen mitochondria with reduction in the number of cristae, pyknotic nuclei with abnormal chromatin condensation (fig. 6). Salvia Rhytidea pre-treatment markedly ameliorated kidney tissue injury and almost restored the normal electron microscopic picture that was observed with the treatment and the sham-operated groups (A,B,E,F,G,H) (fig. 7).

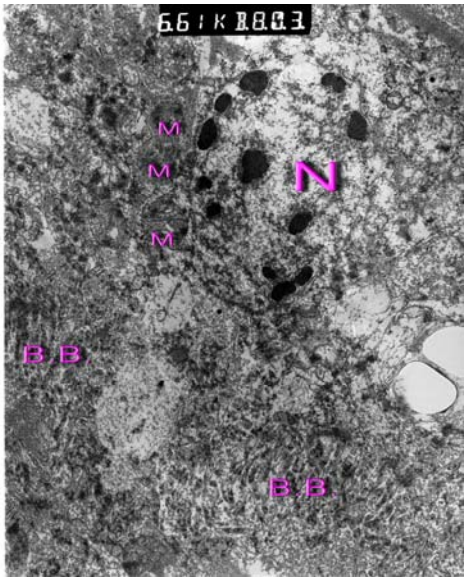

Figure 6. Electron micrograph of a cell of the Rat kidney proximal tubule of the NS/IR group (D): some swollen mitochondria with vacuolated appearance and distorted cristae (M), a pyknotic nuclei with dense clumped marginal chromatin (N), and disordered brush border (BB) are shown . 6600

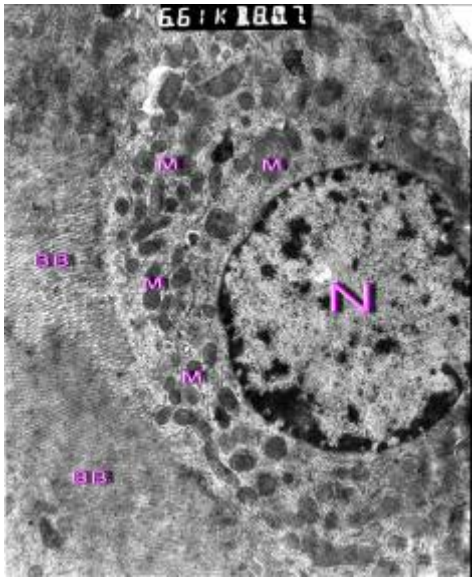

Figure 7. Electron micrograph of rat kidney from the treatment group (B) showing normal appearance of a proximal tubule cell, with smooth rounded nucleus (N), high amount of normally shaped brush border at the surface (BB) and intact mitochondria with normal size, membrane and cristae around nucleus (M). ×6600

Biochemical results

Table 1. BUN and Creatinine in different groups.

	Ischemia Groups*				Sham Groups			
Groups	**A**	**B**	**C**	**D***	**E**	**F**	**G**	**H**
BUN (gr/dl)	30.12 ± 4.35	35.73 ± 5.09	32.45 ± 4.63	106.14 ± 10.32	22.61 ± 3.20	24.16 ± 3.18	23.92 ± 2.82	23.89 ± 4.01
Creatinine (gr/dl)	2.89 ± 0.66	3.35 ±0.71	3.44 ± 0.70	12.81 ± 1.13	1.85 ± 0.42	2.05 ± 0.61	1.93 ± 0.44	1.79 ± 0.51

*Significant difference (P<0.05)

As shown in table 1, both BUN and creatinine increased significantly in ischemia groups in comparisons to sham groups (P<0.05). Maximum amount of BUN and creatinine are in group D, which is the control group, received normal saline, and after 1h ischemia, 24h reperfusion was induced. There is a significant difference between group D, with groups A, B, and C, and all the sham groups (P<0.05).

Discussion

Renal IR injury, which is unavoidable in renal transplantation and is frequently associated with shock or surgery, is a major cause of acute renal failure. [11-14] It has been estimated that ischemic insult, especially during renal transplantation, is responsible for 20–30% of primary graft dysfunction. [15] The classical concept of IR damage was related initially to the ischemic insult with the resulting metabolic alterations due to hypoxia, release of reactive oxygen species (ROS) such as superoxide radical (O_2–), hydrogen peroxide (H_2O_2), and hydroxyl radical (–OH) during reperfusion, neutrophil accumulation, and subsequent release of additional ROS and lytic enzymes. [13,14,16] ROS react with biomolecules such as cell membrane lipid as well as proteins, carbohydrates, nucleic acids, and thiols resulting in organic radical formation, lipid peroxidation, enzyme inactivation, glutathione oxidation, and cell destruction. [2,15,17,18] IR injury may increase the immunogenicity of the organ by upregulating major histocompatibility complex class II antigens, which are associated closely with increased acute rejection episodes.[12,19,20] In addition, in the early transplant period it has been associated with late allograft failure.[20] It also results in decreased glomerular filtration and renal blood flow and increased urine output characterized by natriuresis and impaired concentrating ability.[2]

As shown in histophatological and TEM figures, renal IR resulted in some changes in the tissue which varied from mild epithelial change in group A to severe ATN, thiroidization, hemorrhage and inflammatory cell infiltration in group D. There are many other papers in which the same observations have been reported following renal IR.[1,3,12-15,18,21] As shown in the figures, the injuries after 24 hours is much more severe which is due to developmental nature of IR injuries during the time. Also, the rate of tissue changes and necrosis in treatment groups (A and B), is much less than the control groups (C and D), which shows the positive effect of SR in protecting the kidney from IR injuries. As shown in table 1, the changes in serum BUN and Creatinine are in close correlation with histopathological changes. Serum BUN and Creatinine increased significantly in IR groups in comparison to sham groups, with maximum increase in control group after 24 hours (group D), which shows severe renal dysfunction due to IR injuries. As shown in table 1, though IR resulted in significant increase in BUN and Creatinine in both treatment and control groups, the rate of increase is much less in treatment group, which shows that SR could protect the kidneys' functions significantly.

Changes of BUN and Creatinine following renal IR, have been also reported in some studies.[12,13,15,18]

But how Salvia can exert its protective effects against renal IR injuries? Salvia exhibited activities in modulating vascular tone such as promotion of blood circulation, removal of blood stasis and alleviation of pain.[3,22] It also showed activities in inhibiting platelet aggregation and exerting antioxidant effects.[10] In animal ischemia/reperfusion model, Salvia prevented myocardial infarction injury, decreased the lipid peroxidation[23-25], enhanced antioxidant enzyme activities[10,23,26-29] and scavenged free radicals.[3,10,22-24,26,28,30]

According to the results of this study, it can be concluded that SR has a potent antioxidant properties, through which, can reverse the IR injuries in the kidney.

Acknowledgements

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چکیده:

اسانس مریم گلی ایرانی (*Salvia Rhytidia*) می تواند از آسیب های ناشی از قطع و برقراری مجدد جریان خون در کلیه رت جلوگیری نماید

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هدف- در این مطالعه تأثیر اسانس گیاه مریم گلی ایرانی (لاله زاری) بر کاهش آسیب های ناشی از قطع و برقراری مجدد جریان خون در کلیه رت مورد بررسی قرار می گیرد.
طرح مطالعه- مطالعه تجربی در موجود زنده.
حیوانات- ۴۸ سر موش نر نژاد ویستار با وزن ۲۵۰ تا ۳۰۰ گرم.
روش کار- حیوانات بطور تصادفی به هشت گروه مساوی تقسیم شدند. در گروه های قطع جریان خون (ایسکمی) (گروه های A تا D)، تحت بیهوشی عمومی هردو پایک عروقی کلیوی از رهیافت خط وسط در دسترس قرار گرفته و قطع جریان خون به مدت ۴۰ دقیقه با قرار دادن تورنیکت رومل روی پایک ها القا گردید. گروه های A و B، ۴۸ ساعت، ۲۴ ساعت و یک ساعت قبل از جراحی اسانس گیاهی را بصورت خوراکی دریافت نمودند. در گروه های C و D هم حجم با اسانس گیاهی نرمال سایلین تجویز شد. در گروه های شم (E تا H)، کلیه روشها مانند گروه های A تا D بود با این تفاوت که انسداد پایک عروقی کلیه انجام نگرفت. مدت زمان برقراری مجدد جریان خون در گروه های A، C، E و G یک ساعت و در گروه های B، D، F و H ۲۴ ساعت بود. پس از این زمان به منظور اندازه گیری BUN و کراتینین سرم، خونگیری از قلب حیوانات به عمل آمده و سپس حیوانات برای بررسی های هیستوپاتولوژیک و میکروسکوپ الکترونی قربانی شدند.
نتایج- ایسکمی و برقراری مجدد جریان خون سبب افزایش معنی دار BUN و کراتینین سرم گردید. بیشترین افزایش در گروه D بود که در معرض ۴۰ دقیق ایسکمی و ۲۴ ساعت برقراری جریان خود متعاقب تجویز نرمال سایلین قرار گرفته بود. مشاهدات هیستوپاتولوژی و میکروسکوپ الکترونی نیز دال بر تغییرات بسیار شدیدتر شامل نکروز حاد لوله ای، خونریزی و کبدی شدن، حفره دار شدن سیتوپلاسم، تورم میتوکندریها همراه با کاهش تعداد کریستا ها و هسته های پیکنوتیک بودند.
نتیجه گیری و کاربرد بالینی- این مطالعه نشان داد که اسانس گیاه مریم گلی ایرانی می تواند کلیه ها را در مقابل آسیب های ناشی از قطع و برقرار مجدد جریان خون به کلیه های محافظت نماید، که این تأثیر ناشی از خواص آنتی اکسیدانی گیاه است.
کلید واژگان- قطع و برقرار مجدد جریان خون، کلیه، مریم گلی ایرانی (لاله زاری)، رت.