ABSTRACT

Objective: Use of herbal medicines has increased in recent years and many researches show their values in the treatment and prevention of diseases. In several studies, antioxidant properties of purslane (PO) were demonstrated. The aim of the present study was to investigate the effect of ethanolic extract of PO (EEPO) on the renal function and antioxidant status after induction of ischemia reperfusion (I/R) injury in the rat kidney.

Methods: 30 male Wistar rats were divided into 5 groups (n=6): sham operated+ vehicle (sham), sham operated+ EEPO 300 mg/kg (control), I/R, I/R+ EEPO 150 and 300 mg/kg. Unilateral nephrectomy was performed in all animals, 20 days before the experiment. PO extract or vehicle was administered for 5 days, so that the last dose was administered a half hour before induction of the left kidney I/R (45 min ischemia /24 h reperfusion). At the end of reperfusion period, blood and renal tissue samples were obtained for the serum urea and creatinine (Scr) measurements as well as the tissue antioxidant assays.

Results: Induction of I/R and pretreatment with PO extract, increased the level of superoxide dismutase (SOD). There were no significant changes in the levels of MDA, GSH and FRAP among different groups. On the other hand the Scr and serum urea of the I/R and treated groups were elevated compared to the sham group.

Conclusion: Purslane did not strongly affect the renal antioxidant status and could not prevent the renal injury following I/R. Probable reason might be the stimulation of immune system by the PO extract.

Keywords: Purslane, I/R injury, Renal function, Antioxidant, Rat.

INTRODUCTION

Acute Renal Failure (ARF) is a common and serious clinical syndrome that occurs in many clinical conditions such as "severe hypotension and subsequent resuscitation", "aortoarterial surgeries" and "kidney transplantation" [1]. ARF is associated with high prevalence [2] and despite intensive care; it still leads to a mortality rate above 45% in critically ill patients [3]. It has been shown that I/R injury is a major cause of ARF [4]. A main events responsible for the renal injury during I/R are the production of reactive oxygen (ROS) or nitrogen species (RNS) [5, 6]. These free radicals exert cytotoxic effects, including protein oxidation, lipid peroxidation, DNA damage and induction of apoptosis [7]. The inflammatory response to I/R is another key factor that was demonstrated by complement activation, the generation of cytokines and chemokines within the kidney, as well as infiltration of leukocytes into the kidney [8,9].

The endogenous antioxidants such as Superoxide dismutase (SOD), catalase (CAT) and Glutathione (GSH) are responsible for defense against free radicals and oxidative stress [7]. Imbalance between oxidant/antioxidant systems contributes to the pathogenesis of several disorders including renal I/R injury [7]. So, the use of exogenous antioxidants to prevention or treatment of such diseases has attracted considerable attention [10, 11].

Use of herbal medicines has increased in recent years and many researches show their values in treatment and prevention of diseases including renal I/R [12, 13]. Purslane (Portulaca oleracea L, PO) is one of the most commonly used medicinal plants that is rich in omega-3 fatty acids, gallocateins, kaempferol, quercetin, apigeninan glutathione [14, 15] with apparent antioxidant properties [16, 17]. It has been shown that PO ameliorates oxidative stress in experimental diabetic rats [18]. Also beneficial effects of PO have been shown in conditions such as nephrotoxicity in rat [19, 20].

Therefore, this study was designed to examine the effect of the pre-administration of PO on renal function and oxidative stress in kidney tissues following 45 minutes of ischemia and 24 hours of reperfusion.

MATERIALS AND METHODS

Animals

The study was performed on male Wistar rats, 250–300 g, obtained from the animals' house, faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz. Animals were housed in a ventilated room under a 12/12 hour light/dark cycle at 24±2 °C and had free access to water and food. This study was conducted observing all the ethical codes of working on experimental animals approved by Ministry of Health and Medical Education.

Preparation of extract

Purslane was collected from campus of Ramin University of Ahvaz and was identified by Department of Pharmacology, University of shahid chamran, Ahvaz. The aerial parts of Purslane were cleaned, dried in shadow and powdered by mechanical grinder. For preparing ethanolic extract by maceration method, 300 grams of dry powder was poured in a sealed glass container and 500 ml of ethanol was added to it. After 72 hours, the mixture was centrifuged and then it was put in a bath of warm water to complete evaporation of its alcohol. Afterward, the extract was concentrated in vacuum to the eligible volume.

Experimental protocols

Thirty male Wistar rats were randomly divided into 5 groups (n=6). Sham and I/R group received normal saline (vehicle), treatment groups received 150 and 300 mg/kg of PO extract (EEPO150+ I/R, EEPO300+ I/R), and control group received PO extract at 300 mg/kg (EEPO300). EEPO or vehicle was administered for 5 days, so the final dose was administered a half hour before induction of ischemia. All vehicles and treatments set at the same volume (4.5 ml/kg) and were administered orally. Right kidney nephrectomy was performed in all groups, 20 days before the experiment. In the sham and
EEPO300 groups all surgical procedures were done except clamping of the renal pedicles. On the day of the experiment, animals were anaesthetized by Ketamine (80mg/kg) and xylazine (10mg/kg). Body temperature was recorded rectally and maintained at 37°C. After shaving the abdominal hair, a longitudinal incision was made and the left renal pedicle was isolated. After a 30 min stabilization period, I/R injury was performed by clamping of the left renal Pedicle for 45 min. Clamp was removed after 45 min then abdominal wall was closed with 3-zero silk. After 24 hours reperfusion period, animals were anesthetized again and blood samples were obtained from aorta then centrifuged and serum samples were kept in -20°C until measurement of the serum creatinine and urea.

The left kidney was removed and immediately washed with normal saline and stored at -70 °C for the measurement of MDA, GSH, and SOD levels as well as the total antioxidant capacity (FRAP).

Renal function assays

The level of serum creatinine was measured according to the manual of the commercial kit (Pars Azmon Co. Karaj, Iran) Enzymatically. The level of Scr was measured according to the manual of the appropriate kit (Pars Azmon Co. Karaj,Iran) using the Jaffe method.

SOD, GSH, MDA and FRAP assays

The kidney tissues were weighed and homogenized in phosphate buffer (1:10 pH = 7.4) on ice by homogenizer device (Micra, Germany). The homogenates were centrifuged in 12000 ×g for 15 minutes at 4°C and supernatants were separated and used for SOD, MDA, GSH and FRAP assays. The total SOD activity was measured according to the manual of Randox kit (Randox Lab, BT29QY, UK). Tissue GSH level was measured by Elman’s method [21]. Degree of lipid peroxidation in kidney tissue homogenates was determined according to the method of Buege and Aust [22]. Measurement of FRAP had done spectrophotometrically by one of the most common methods for evaluation of total antioxidant activity [23].

Statistical analysis

Statistical analysis was carried out using SPSS software. Results are shown as mean ± SEM. Groups of data were compared using one-way analysis of variance (ANOVA) followed by LSD test. A P<0.05 was considered as statistically significant.

RESULTS

Renal function

As shown in the fig.1, induction of I/R elevated the Scr almost three folds. Pre-treatment with EEPO had no significant effect as shown by comparison between I/R and treatments groups. The relatively same results were obtained for serum urea (Fig.2). I/R and treatment groups had higher levels of this parameter significantly. Administration of EEPO alone did not change serum urea or creatinine concentration (Fig.1 and 2).

MDA, GSH, SOD and FRAP

After induction of I/R the MDA level increased but not significantly. Alterations of it in other groups were not significant too. The GSH and FRAP as indicators of antioxidant status of the renal tissue did not show any significant changes (Table 1). As shown in the table, administration of PO extract at the dose of 300 mg/kg in control group and induction of I/R increased SOD (p<0.05). SOD continued to rise in the treatment groups as indicated by their comparisons with the sham group (p<0.01).
Production of free radicals and reactive oxygen species (ROS) is and non-enzymatic antioxidants are responsible for protection of tocopherol, vitamin A and vitamin C [26]. Glutathione peroxidase, catalase (CAT), glutathione (GSH), vitamin E, and others against oxidant stress, such as superoxide dismutase (SOD), glutathione peroxidase, catalase (CAT), glutathione (GSH), vitamin E (tocopherol), vitamin A and vitamin C [26].

Renal I/R injury is a complex and multifactorial phenomenon [24]. Production of free radicals and reactive oxygen species (ROS) is postulated to have a key role in I/R injury [25]. Several enzymatic and non-enzymatic antioxidants are responsible for protection of tissues against oxidant stress, such as superoxide dismutase (SOD), glutathione peroxidase, catalase (CAT), glutathione (GSH), vitamin E (tocopherol), vitamin A and vitamin C [26].

The potent antioxidant activity of PO showed in some previous studies [27, 28]. In addition benefits of its use in different renal diseases were indicated [19, 20, 29]. Karimi et al. reported that aqueous and ethanolic extracts of PO could ameliorate the alterations of BIUN, serum Cr and renal histology in rats exposed to ciprofloxin-induced renal toxicity [19]. Hozayan et al. examined the effects of aqueous extract of PO on gentamicin nephrotoxicity in Albino Rats. They indicated that GO-administration of PO extract and fish oil improve the adverse changes in the kidney functions by an increase in antioxidants activities and reduction of peroxidation [20]. Lee et al. also reported that the aqueous extract of PO ameliorated diabetic nephropathy through suppression of fibrosis and inflammation in the kidney [29].

The results of this study showed that the PO extract increased SOD in treatment groups as well as control (EEPO300) and I/R groups. Although MDA, GSH and FRAP remained approximately unchanged. These findings probably in somehow be due to the methods used in the experiment. In this study all animals experienced right nephrectomy 20 days before induction of I/R. It has been shown that unilateral nephrectomy causes compensatory changes in remaining kidney [30, 31]. Kietklin et al. indicated that inflammation markers were only altered for less than 24 h after unilateral nephrectomy and they return to baseline gradually [32]. The compensatory changes in the function of remaining kidney have been shown by increasing glomerular filtration rate and effective renal plasma flow after unilateral nephrectomy in sheep [33].

In the present study we observed the significant alterations in serum urea and Scr but not in the oxidant and most of antioxidant markers following I/R induction. One possibility might be the time of the measurements (24 hours after reperfusion period). In agreement to this idea Junor et al. reported that MDA content rose strikingly at 5 minutes of reperfusion and returned near to normal levels 24 hours later [34]. In addition, previous studies revealed that urea and Cr levels remain high for the long time following I/R injury [35, 36].

In the present study SOD was the only antioxidant that showed significant changes. This is in agreement with previous studies that reported the elevation in SOD activity following renal IR injury [37, 38]. This finding may be related in part to the more stability and long half life of this antioxidant [39]. On the other hand Dobashi et al. reported that expression of antioxidant genes during renal ischemia-reperfusion are not coordinately expressed [40]. They observed that 60 or 90 min of ischemia followed by 0, 2 or 24 h of reperfusion resulted in a decrease in the renal antioxidants such as catalase, glutathione peroxidase and SOD, but MnSOD activity tended to recover towards normal during reperfusion period. They also noticed that the mRNA for MnSOD was upregulated at all time points of ischemia-reperfusion injury [40].

Interestingly, we observed that SOD increased not only in treatment groups but also in I/R and control groups. It seems that PO extract exerted a stimulatory effect on SOD activity. Ming Yin et al. in their study indicated that SOD gene and its transduction minimized ischemia-reperfusion-induced acute renal failure [41]. Some other studies also reported the beneficial effects of SOD in prevention of oxidative stress in the kidney and other organs [42, 43], but in the present study, in spite of SOD elevation, we did not observe protective effect in renal function of the treatment groups upon their serum urea and Cr increments.

One important mechanism of the I/R injury is the inflammatory response that starts upon reperfusion following ischemia [44]. In this inflammatory response, macrophages, endothelial cells, neutrophils, lymphocytes, platelets, parenchymal cells, as well as noncellular elements may be involved [24]. Kadkhodaee et al. have shown that even transfer of leucocytes from mice exposed to renal I/R to the non-ischemic mice, induced renal oxidative damage [45]. Some previous studies suggest that PO extract has modulatory effects on immune system. Oyedeji et al. indicated that treatment of rats with 1 mg/kg and 2 mg/kg of PO fraction caused significant increase in total white blood cell and lymphocyte count [46]. Another study reported that PO polysaccharides increase spleen, thymocyte T and B lymphocyte proliferation [17]. Then the stimulatory effect of PO on immune system could be one possible reason for our observations in this study.

Production of nitric oxide (NO) and its metabolites is another postulated mechanism of I/R injury [47]. The role of NO in pathogenesis of I/R injury has been controversial because of the complexity of NO synthase (NOS) isoforms [48]. The generation of NO by inducible NOS synthase (iNOS) has been related to rennal cell injury due to infiltration of inflammatory cells, by direct DNA damage or by apoptotic effects. On the other hand, a reduction in endothelial NOS (eNOS) activity contributes to renal impairment due to endothelial dysfunction and consecutive renal vasocostriction [49]. On the other hands, it has shown that NO blocks leukocyte infiltration and prevents deleterious effects of activated leukocytes on renal function [48]. Abas et al. reported that PO extract inhibited NO production significantly in a concentration-dependent manner [50]. Although the results of above study postulated that PO blocked iNOS but it seems that it could not effectively inhibit the NO production in the present study. One possible reason might be the stimulatory effect of PO extract on immune system and more iNOS activity due to it. Further studies are required to identify the exact mechanisms of the effects of PO extract on the renal I/R injury.

CONCLUSION

Despite powerful antioxidant properties of PO extract, it could not protect kidney from IR injury by doses and methods that have been used in the present study. Probable reasons might be the stimulation of the immune response and more NO production by iNOS.
CONFLICT OF INTERESTS
Declared None

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