

Original Article

Effect of purslane on kidney failure following copper toxicity in a rat modelAbdollah Ramzani Ghara¹ Fereshteh Ezzati Ghadi^{2*}

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Abstract

Background and purpose: Copper (Cu) is an essential trace element. The toxic level of copper can catalyze the formation of free radicals which cause various diseases including kidney failure. The main aim of this study was to evaluate the effects of purslane on kidney failure due to copper toxicity in rat model.

Materials and Methods: Twenty-eight male Wistar rats were divided equally and randomly into four groups. Group I was control group, while in group II, copper sulphate was administrated orally in dose of 200 mg/kg body weight every day for one month. In group III, on the other hand, purslane was orally given in a dose of 400 mg/kg body weight per day for one month. Group IV received combined treatment of copper sulphate and purslane as described in groups II and III. Blood urea nitrogen (BUN) and serum creatinine was then measured. The kidney tissues were subject to histopathological study.

Results: The results showed that serum BUN and creatinine were increased in the copper-treated rat which were $52/20 \pm 4/91$ and $0/56 \pm 0/06$, respectively. Purslane administration also decreased the elevated level of creatinine and BUN in rats which received toxic levels of copper ($0/48 \pm 0/03$ and $44/80 \pm 5/7$, respectively).

Conclusion: The present study revealed that purslane improved some kidney function parameters due to its antioxidant and anti-inflammatory properties.

Key words: Blood urea nitrogen; Copper toxicity; Purslane; Creatinine

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1. Introduction

During the last few decades, increased anthropogenic activities, rapid industrialization, and modern agricultural practices have resulted in increased heavy metal contamination in the environment, which causes toxicity to the living organisms (1-3). Large areas of land have been contaminated with heavy metals due to the use of pesticides, fertilizers, municipal, compost wastes, as well as heavy metal release from smelting industries and metalliferous mines (4).

Metal ions that enter the body are biologically active, participating in a variety of different physiological and pathophysiological reactions. Another negative consequence of heavy metal accumulation is the generation of reactive oxygen species known as ROS. ROS are unstable, highly reactive, and thus, promptly react with other macromolecules to generate more free radicals (5). Being extremely reactive in nature, ROS can interact with macromolecules, such as DNA, pigments, proteins, lipids, and other essential cellular molecules depending on the properties like chemical reactivity, redox potential, half-life, and mobility within the cellular system, ultimately leading to a series of destruct in processes collectively termed as “oxidative stress” (6,7). Copper is an essential trace element and has numerous functions in cellular biochemistry (8). In the serum, most of the copper (65–90%) binds with ceruloplasmin, and the rest of that binds with albumin, transcuprein, and amino acids. Excessive amount of free Cu resulted in intracellular and extracellular Cu deposition in various organs. Free copper is able to produce hydroxyl radicals and, therefore, cellular injury (9). The findings

of the study showed that high level of copper can accumulate in different organs, such as liver and kidney (10). The elevated level of copper in different organs can also induce the oxidative stress. The growing body of evidence suggests that plant compounds have protective effect on oxidative stress-induced different diseases, such as cardiovascular disease, cancer, and other diseases (11,12). *Portulaca oleracea* L. belongs to *Portulacaceae* family, which refers to the common purslane, and is known as Khorfeh in Persian, and is listed in the World Health Organization as one of the most used medicinal plants. Purslane contains many compounds, including alkaloids, such as reddish betacyanins and yellow betaxanthins, omega-3 fatty acids, vitamins (mainly vitamin A, vitamin C, and some vitamin B, and carotenoids), as well as dietary minerals, such as magnesium, calcium, potassium, and iron. It is also rich in coumarins, flavonoids, polysaccharide, cardiacglycosides, and anthraquinone glycosides (13,14). Many studies have demonstrated various pharmacological effects of this plant including hypoglycaemic, hypocholesterolemic, and antioxidant effects (15,16). The main aim of the present study was to evaluate the protective role of purslane against free radical-induced kidney failure.

2. Materials and Methods

2.1. Plant preparation

Fresh leaves from purslane herb were collected in Jiroft – a city in Iran – in August 2016. The whole plant leaves were collected and washed in running tap water. An aqueous juice of the purslane herbs were prepared by mashing in a proportion of 1:5 (w/v) and were left for about 24 hours.

After mashing, the resulting crude extract was filtered and dried in a bath of warm water.

2.2. Animals and experimental design

Twenty-eight male Wistar rats of 180-200 g weight were purchased from Razi Research Institute of Kerman, Iran and kept in the Central Animal House, Department of basic sciences, University of Jiroft. The rats were housed in groups of seven per cage and maintained under standard laboratory conditions (12 h light: 12 h dark, and $24 \pm 2^\circ\text{C}$) during the experimental period. Free access to food and water was also allowed at all times. All experimental protocols used on the animals were done with the approval of the Guiding Principles for the Care and use of Research Animals, and were approved by the Animal Ethics Committee at the department of basic sciences, University of Jiroft, Iran. Then, the animals were randomly and equally assigned into four treatment groups. Animals in Group I were served as normal controls and were given water and diet *ad libitum*. Animals in Group II were given copper orally in the form of copper sulphate pentahydrate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) (by gavage) every day at 200 mg/kg body weight for four weeks (17). Group III animals were given purslane at 400 mg/kg body weight every day (by gavage) for four weeks (18). Animals in Group IV were given a combined treatment of copper sulphate, as well as purslane, similarly to Group II and Group III animals, respectively. Animals from all of the groups were then sacrificed by cervical dislocation under light ether anesthesia at the end of the study.

2.3. Biochemical estimations and processing of kidney tissue

At the end of the study, the rats were sacrificed by cervical dislocation under

light ether anesthesia 12 h after receiving the last treatment. The blood samples were collected and allowed to clot and the serum was separated by centrifugation at $3000 \times g$ for 10 min. Serum was then used to estimate creatinine and BUN.

The enzymatic method based on urease was used to measure BUN (Pars Azmoon Inc, Tehran, Iran). Creatinine concentration was then determined using spectrophotometric assays (Pars Azmoon Inc, Tehran, Iran).

For the evaluation of histopathological studies, kidney from each animal was fixed in buffered 10% formalin, preparing the blocks in paraffin, and the sections of about $7\mu\text{m}$ thickness were stained with hematoxylin and eosin (H&E) according to the method of Humanson (19).

1.4. Statistical analysis

The collected data were analyzed statistically, using SPSS Version 19. One-way analysis of variance (ANOVA) was performed to compare the different groups and to determine the overall effect of each treatment, followed by a multiple post hoc test, while the least significant difference of 5% ($p < 0.05$) was considered as significant. The results are represented as mean \pm standard deviation.

3. Results

3.1. Serum biochemical estimations

Table 1 depicts the biochemical estimation in different treated groups in rats. The results showed that creatinine and BUN experienced a significant increase ($p < 0.01$) in the copper-treated rat. Moreover, the data analysis revealed that purslane administration decreased the BUN and creatinine level in the combined treatment of purslane + copper sulphate.

Table 1. Serum biochemical estimation of Purslane-treated rats with kidney failure

Groups	Serum biochemical estimation	
	Creatinine (mg/dl)	BUN(mg/dl)
I	0/46 ± 0/05	43/20± 1/30
II	0/56± 0/06 ^b	52/20± 4/91 ^b
III	0/45± 0/06	42/60± 3/20
IV	0/48± 0/03 ^x	44/80± 5/71 ^y

N=7, results are presented as mean ± SD. Group I: control animals, group II: copper sulphate-treated rats, group III: purslane treated animals, and group IV: combined treatment of copper sulphate + purslane-treated rats. ^b $p < 0.01$ represented the statistical significant between control animals and all treated groups. ^x $p < 0.05$ and ^y $p < 0.01$ also showed the significant difference between group II and IV.

3.2. Histopathological study

The results of cross-sections of kidney tissues from different groups of treated rats are provided in Figure 1. As is shown in the figure (A), the kidney showed normal

glomerular structure. The kidney failure was also associated with marked histological changes, such as glomerulosclerosis, tubular epithelial hypertrophy, glomerular basement membrane wrinkling, and dilation of the urinary space, which were revealed in the copper-treated animals (Figure 1 (B)). In the group treated with combined treatments of aqueous extract of purslane+copper sulphate, the symptoms improved to some extent, so that the lower urinary tract, increased glomerular diameter, and normal tubules were observed (Figure 1 (D)).

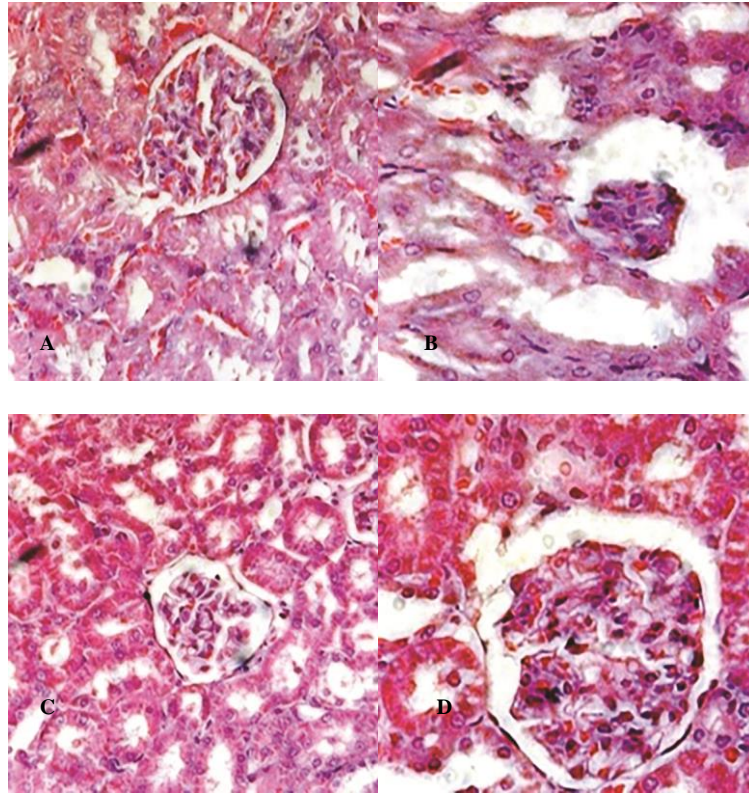


Figure 1. Representative Photomicrographs of Hematoxylin & Eosine stained kidney

Sections. A) Control group showing normal renal tubes and glomerul. B) Rats treated with copper sulphate and showing the glomerulosclerosis. C) Purslane treated rats with normal glomerular structure. D) Purslane + copper treated rats. This group showed increased glomerular diameter and normal tubules. Original magnification \times 400.

4. Discussion

This study revealed kidney failure following toxic level of copper sulphate. A number of studies have shown that copper toxic level can cause kidney failure (20,21). In the present study, copper toxic level produced a marked increase in serum blood urea and creatinine. Studies show that high dose of Cu is associated with an increasing level of creatinine and BUN of serum which is then in accordance with the findings of this report (22). In a study, the toxic level

of Cu-induced kidney damage was examined, and the serum biochemical changes of kidney correlated with MDA level as oxidative stress marker. Also, an elevated level of MDA was positively correlated with BUN and serum creatinine (17). The previous study of the researchers in the same lab showed that the high dose of Cu induced liver necrosis, and the serum MDA level increased in the copper-treated rats (23). Thus, the elevated level of BUN and creatinine in this study can be explained by the increasing level of MDA and oxidative stress.

In the present study, the protective effect of purslane on kidney failure was further investigated by histopathological study. Few studies reported the histopathological changes following copper toxicity (24). The current study showed the kidney damage in copper-treated animals, and revealed that aqueous extract of purslane has protective effect on renal injury. It could be due to the

antioxidant activity of purslane. Moreover, similar dose of purslane was used previously for its antioxidant effect on copper sulphate induced liver necrosis (25). It has also earlier reported to exhibit antioxidant and hepatoprotective activity (26,27). These findings were found to be in line with other scholars' results (22,28,29). Several researchers reported the antioxidant activity of purslane. The antioxidant and free radical scavenging properties of the purslane have earlier been demonstrated and attributed to its constituents, like phenolic containing flavonoides, alkaloids, omega-3, and glutathione (30-32). Purslane co-administration with fish oil was used to treat the nephrotoxicity by gentamicin (33). Purslane contains α -linoleic acid which acts against oxidative stress (34). The findings of the study showed that phenolic compounds have potential oxidative stress inhibition in medicinal plants (35). Antioxidant activity of various plants is also associated with their total phenol and total flavonoid content (36). It was found that purslane possesses hepatoprotective effects by inhibiting oxidative stress, enhancing antioxidant level, and depleting MDA level (37). Several studies showed that the aqueous extract of purslane possesses marked nephroprotective activity, and have a promising role in the treatment of acute renal injury induced by nephrotoxins (38,39).

Overall, it can be concluded that copper sulphate induced kidney changes were mainly due to oxidative stress. Moreover, purslane as an antioxidant source protects tissue from free radicals and can be used for the treatment of kidney failure induced by toxic level of copper sulphate.

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Conflict of interest

No potential conflict of interest relevant to this article was reported.

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