

## Preventive Effect of Garlic Juice on Renal Reperfusion Injury

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**Introduction.** Renal reperfusion injury is associated with increased mortality and morbidity due to acute kidney failure. Oxidative stress induced with renal reperfusion affects glomeruli and tubular epithelium through reactive oxygen species; therefore, the use of medicinal plants appears rational for improvement of reperfusion effects. The aim of present study was to examine the preventive effect of garlic juice (*Allium sativum*) on renal reperfusion injury in rats.

**Materials and Methods.** A total of 30 male Wistar rats were divided into 5 groups: control, garlic, sham (right nephrectomy), reperfusion, and reperfusion + garlic groups. After right nephrectomy, renal ischemia and reperfusion were induced. At the end of the experiment, all rats were killed and kidney function tests and histopathological examination were performed.

**Results.** Reperfusion increased serum urea and fractional excretion of sodium levels, while it decreased urine potassium levels and creatinine clearance. However, garlic juice significantly decreased serum urea levels in the reperfusion + garlic group compared with the reperfusion group ( $P < .001$ ). Pretreatment with garlic juice also resulted in significant increase in urine potassium ( $P = .03$ ) compared to reperfusion. Fractional excretion of sodium and creatinine clearance were also improved. On histological examination, rats pretreated with garlic juice had nearly normal morphology.

**Conclusions.** The results of this study showed that garlic juice significantly prevented renal reperfusion-induced functional and histological injuries.

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## INTRODUCTION

Renal reperfusion injury is common in several clinical situations including kidney transplantation, hemorrhagic shock, major vascular surgery, and certain hypotensive states.<sup>1-3</sup> Renal ischemia induces oxidative stress, which results in aggravated and prolonged systemic inflammatory response after reperfusion. This issue has been studied in animal and clinical models.<sup>1,2</sup> Reperfusion injury is one of the main causes of acute kidney injury, which can manifest histologically as acute tubular necrosis.<sup>4</sup> Reactive oxygen species are generated in high concentration in ischemic organs after

reperfusion. Increased reactive oxygen species directly compromises glomerular and tubular epithelium integrity, one of the factors in the development of acute tubular necrosis.<sup>5</sup>

Under normal conditions, naturally occurring antioxidant enzymes counteract the cellular effects of oxygen-free radicals. During reperfusion of an ischemic organ, the protective ability of these scavengers is overwhelmed by rapid generation of reactive oxygen species, which results in cell death by necrosis and apoptosis.<sup>6</sup> However, during reperfusion injury and similar conditions of oxidative stress, accumulation of reactive

oxygen species, reductions in antioxidant enzymes expression/activities, or a combination of both lead to profound damage to cellular components such as DNA, proteins, and lipids.<sup>3,7</sup> Complement proteins, chemokines, and adhesion molecules are also known to play an active role in the development of renal reperfusion injury.<sup>2</sup>

Recent overwhelming attention to herbal and alternative medicine has encouraged plant chemists, pharmacologists, biochemists, and molecular biologists to combine their efforts in search for natural agents that can limit free radical-mediated injuries during and following ischemia and reperfusion, for better therapeutic management of reperfusion injuries. Several plant-derived agents have been reported to afford protection against renal reperfusion injury, including the herb extracts from saffron, proanthocyanidin-rich extracts from grape seeds, *Ginkgo biloba* extract, and *Coptidis rhizoma* extract. *Coptidis rhizoma* provides protection by scavenging NO•, O<sub>2</sub>•<sup>-</sup> and ONOO<sup>-</sup> generated during renal reperfusion, and rehmanna glutinose, and lithospermic acid B, isolated from *Salvia miltiorrhiza*, have all ameliorated renal reperfusion injury in rats.<sup>8-13</sup>

Garlic (*Allium sativum*) is a widely cultivated plant with both culinary and medicinal uses stemming from its proposed biological activities, which include anticancer, antibiotic, anti-thrombotic, lipid-lowering, and cardiovascular effects.<sup>14,15</sup> Garlic in different forms has antioxidant properties. These properties are shown to be due the existence of compounds such as water soluble organosulfure compounds, S-allylcysteine, and lipid soluble compounds like diallyl sulfide.<sup>16,17</sup> According to the protective effect of garlic in treatment of reperfusion injury in other studies, the aim of present study was to assess the preventive effects of garlic juice on renal reperfusion injury in rats.

## MATERIALS AND METHODS

### Study Rats

Thirty male Wistar rats, weighting 230 g to 260 g, were used throughout the study. The animals were housed under standard conditions of light and dark cycle with free access to food and water. The rats were divided into 5 groups of 6: control group, with no treatment and no operation; garlic group, which only received garlic juice 24 hours and immediately before putting in the metabolic

cage; sham group, in which only surgery (right nephrectomy) was done without induction of ischemia; reperfusion group, which were exposed to unilateral ischemia and reperfusion (45 minutes of left renal pedicle occlusion followed by 24 hours of reperfusion); reperfusion + garlic group, in which animals received garlic juice for 24 hours and immediately before surgical procedure.

### Surgery and Experimental Design

Rats in reperfusion, reperfusion + garlic, and sham groups were anaesthetized with intraperitoneal injection of ketamine-xylazine (50 mg/kg and 10 mg/kg, respectively). The abdominal area was prepared with povidone iodine, a midline incision was made, and right nephrectomies were performed. In the two groups with reperfusion, ischemia was induced with left renal pedicle clamping with a vascular clamp for 45 minutes. After removing the clamp, the abdomen was closed in 2 layers. In all groups, the animals were kept in metabolic cages for 24 hours to collect urine and also to measure water consumption. At the end of the 24 hours, the rats were killed by decapitation, and the blood samples were obtained and immediately centrifuged to collect sera. Serum and urine samples were stored at -20°C until analysis. The left kidney from each rat was also harvested and weighted for measurement of the ratio of kidney weight to body weight and histopathological examination.

### Preparation and Administration of Garlic Juice

Fresh garlic bulbs were cut into small pieces. The bulbs were crushed in a mixing machine, and 250 mL of distilled water per 50 g of garlic was added. The resultant slurry was squeezed and filtered through a fine cloth and the filtrate was quickly frozen until used. The garlic and the reperfusion + garlic groups received 1 mL of garlic juice per 100 g of body weight by gavage. Other groups only received distilled water.

### Biochemical Analysis

Creatinine and urea concentrations of serum and creatinine of urine were measured spectrophotometrically by using commercial kits (Parsazmun, Tehran, Iran) by an autoanalyzer instrument, and creatinine clearance was calculated. Serum and urine sodium concentrations were measured by a flame photometer instrument,

and then fractional excretion of sodium was calculated. Urine potassium was measured by a flame photometer instrument. Urine was diluted 1 to 100 by distilled water for measurement of urine sodium and potassium. Creatinine clearance and fractional excretion of sodium were calculated as follows<sup>18</sup>:

Creatinine clearance = urine creatinine × urine volume/plasma creatinine

Fractional excretion of sodium = (urine sodium /plasma sodium) × (plasma creatinine/urine creatinine) × 100

### Histopathological Studies

The kidneys were processed for light microscopic observation, according to standard procedures. The kidneys were fixed in 10% natural buffered formalin and embedded in paraffin. Tissue sections of 3 μm were obtained and stained with hematoxylin-eosin. Histopathological studies were performed under a light microscope. All specimens were examined for 7 histological parameters including cellular vacuolation, apoptosis, interstitial edema, tubular dilatation, hyaline cast, polymorphonucleocytes in outer medulla, and medullary congestion on a semiquantitative scale of none (-), mild (+), moderate (++) , and severe (+++).<sup>19-21</sup> Tubular epithelial necrosis was graded as follow: normal histology (-), tubular cell swelling and nuclear condensation with up 1/3 of tubular profile exhibiting nuclear loss (+), tubular cell swelling and nuclear condensation with 1/3 to 2/3 of tubular profile exhibiting nuclear loss (++) , greater than 2/3 of tubular profile showing nuclear loss (+++).<sup>21,22</sup>

### Statistical Analyses

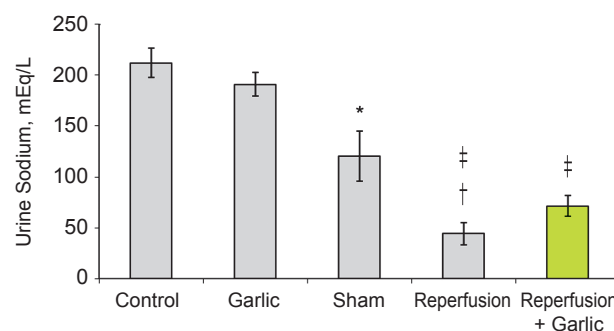
Data were expressed as mean ± standard error of mean. Statistical analysis was performed using the 1-way analysis of variance followed by the Tukey post-hoc test. Differences were considered significant at *P* values less than .05.

## RESULTS

### Biochemical Findings

Animals that underwent renal ischemia-reperfusion exhibited significant increase in serum concentrations of urea levels as compared with other groups (*P* < .001) and in serum creatinine levels versus the control, garlic, and sham groups (*P* < .001) and the reperfusion + garlic group (*P* = .005). Pretreatment with garlic juice significantly decreased serum creatinine and urea levels compared with the reperfusion group (Table 1).

In groups with reperfusion induction, urinary creatinine significantly decreased compared to the sham group (*P* < .001). Urine sodium also decreased more than that in the control and garlic groups (*P* < .001) and the sham group (*P* = .02). Urine potassium also significantly decreased, as compared with that in the control and garlic groups (*P* < .001), the sham group (*P* = .01), and the reperfusion + garlic group (*P* = .03). Pretreatment by garlic juice increased urinary creatinine, sodium, and potassium, as compared with the reperfusion group (Table 1 and Figures 1 and 2).



**Figure 1.** Effect of garlic juice pretreatment on urine sodium in rat groups. In the reperfusion group, urine sodium is lower than other groups. Urine sodium level increases in the reperfusion + garlic group.

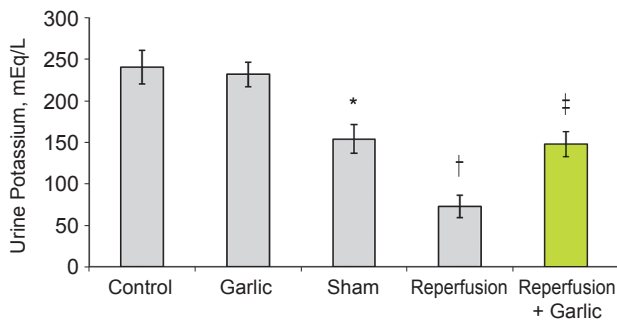
\**P* = .03 versus control + garlic group and *P* = .003 versus control group.

†*P* = .02 versus sham group.

‡*P* < .001 versus control and garlic groups.

**Table 1.** Effect of Garlic Juice Pretreatment on Serum and Urine Creatinine, Serum Urea, and Fractional Excretion of Sodium (FENa) in Rats Exposed to Renal Reperfusion

Parameters	Experimental Rat Groups				
	Control	Garlic	Sham	Reperfusion	Reperfusion + Garlic
Serum urea, mg/dL	46.5 ± 2.6	42.8 ± 1.6	55.0 ± 4.8	200.8 ± 28.8	72.7 ± 5.3
Serum creatinine, mg/dL	0.82 ± 0.04	0.76 ± 0.05	0.84 ± 0.035	1.87 ± 0.03	1.12 ± 0.09
Urinary creatinine, mg/L	60.3 ± 11.8	62.2 ± 6.3	92.9 ± 13.3	18.5 ± 7.5	55.1 ± 7.4
FENa, %	1.68 ± 0.18	1.44 ± 0.13	0.51 ± 0.11	3.97 ± 1.20	0.99 ± 0.27



**Figure 2.** Effect of garlic juice pretreatment on urine potassium in rat groups. In the reperfusion group, urine potassium is lower than other groups. Urine potassium level increases in reperfusion + garlic group.

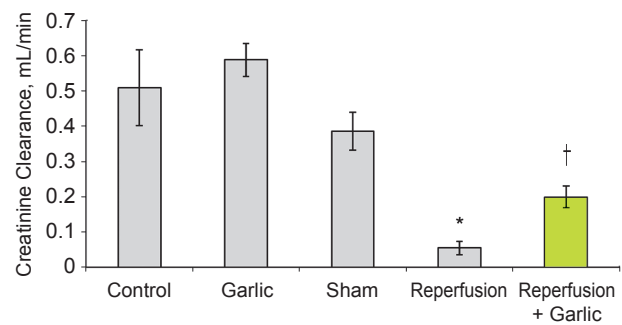
\* $P = .02$  versus control + garlic group and  $P = .007$  versus control group.

† $P = .01$  versus sham group,  $P = .03$  versus reperfusion + garlic group, and  $P < .001$  versus control and garlic groups.

‡ $P = .004$  versus control group and  $P = .01$  versus garlic group.

Reperfusion injury decreased creatinine clearance as compared with creatinine clearance in the control and garlic groups ( $P < .001$ ) and the sham group ( $P = .003$ ). Also, reperfusion increased fractional excretion of sodium compared with that in all other groups (control,  $P = .046$ ; garlic,  $P = .02$ ; sham,  $P < .001$ , and reperfusion + garlic,  $P = .002$ ; respectively). Both creatinine clearance and fractional excretion of sodium were significantly reversed by oral administration of garlic juice (Figure 3 and Table 1).

Urinary excretion rate significantly increased in animals with reperfusion injury as compared with the sham and reperfusion + garlic animals ( $P < .001$ ). It decreased by pretreatment of garlic juice in reperfusion + garlic group (Figure 4). Water intake increased in animals with reperfusion injury as compared with rats in the reperfusion + garlic group ( $P = .007$ ) and sham animals, and it decreased by pretreatment of garlic juice in the reperfusion + garlic group (Table 2). Finally, the ratio of kidney weight to body weight also increased in the reperfusion group as compared



**Figure 3.** Effect of garlic juice pretreatment on creatinine clearance in rat groups. In the reperfusion group, creatinine clearance is significantly lower than other groups. Creatinine clearance level increases in the reperfusion + garlic group.

\* $P = .003$  versus sham group and  $P < .001$  control and garlic groups.

† $P = .007$  versus control group and  $P = .001$  versus garlic group.



**Figure 4.** Effect of garlic juice pretreatment on urinary excretion rates in rat groups. In the reperfusion group, urinary excretion rate is higher than other groups. Urinary excretion rate decreases in the reperfusion + garlic group.

† $P = .03$  versus control group and  $P = .04$  versus garlic group, and  $P < .001$  versus sham and reperfusion + garlic groups.

\* $P < .001$  versus control and garlic groups.

with control, garlic, and sham groups ( $P < .001$ ). Pretreatment of garlic juice decreased this ratio compared with no garlic juice in the reperfusion group (Table 2).

### Histopathological Findings

Microscopically, reperfusion group showed significant pathologic changes of tubular epithelial necrosis. Low tubular epithelial necrosis scores

**Table 2.** Effect of Garlic Juice Pretreatment on Water Intake and Kidney-body Weight Ratio in Experimental Rats

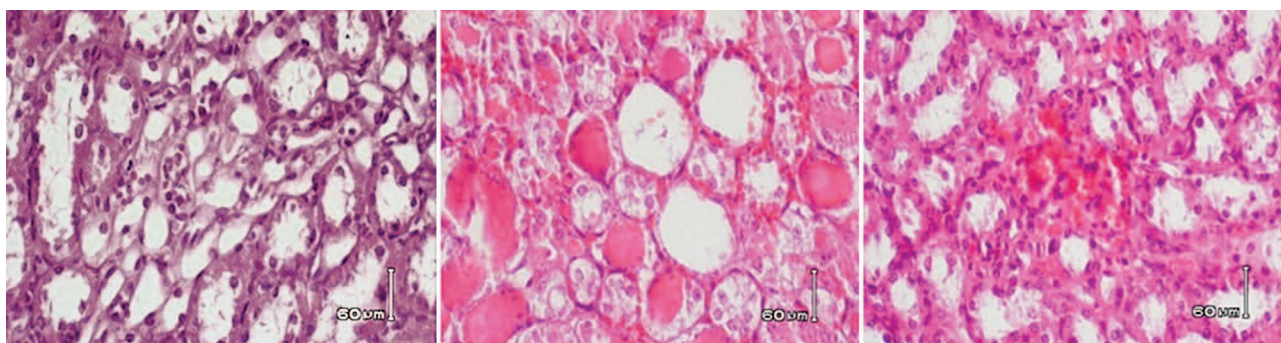
Parameters	Experimental Rat Groups				
	Control	Garlic	Sham	Reperfusion	Reperfusion + Garlic
Water intake, mL	35.5 ± 2.8	37.0 ± 4.3	12.1 ± 0.9*	20.9 ± 2.2†	8.9 ± 0.9*
Kidney-body weight ratio	0.0036 ± 0.0000	0.0037 ± 0.0001	0.0040 ± 0.0001‡	0.0046 ± 0.0001§	0.0043 ± 0.0001*

\* $P < 0.001$  versus control and garlic groups.

† $P = .002$  versus control group,  $P = .001$  versus garlic group, and  $P = .007$  versus reperfusion + garlic group.

‡ $P = .008$  versus control group and  $P = .02$  versus garlic group.

§ $P < .001$  versus control, garlic, and sham groups.



**Figure 5.** Sections of the rats' kidneys (hematoxylin-eosin,  $\times 40$ ). **Left**, Kidney section of a rat in the sham operation group shows control tubules. **Middle**, Kidney section of a rat exposed to left renal pedicle ischemia and reperfusion shows congestion surrounding the tubules. Tubular epithelial necrosis, vacuolation, and hyaline cast are also apparent. **Right**, Kidney section of a rat with reperfusion injury pretreated with garlic juice, in which morphology is nearly normal except for mild congestion.

**Table 3.** Histopathological Changes of Kidneys in Rats

Feature	Experimental Rat Groups			
	Garlic	Sham	Reperfusion	Reperfusion + Garlic
Tubular epithelial necrosis score	-	-	+++	+
Polymorphonucleocyte in outer medulla	-	-	+	-
Cellular vacuolation	-	-	+++	+
Interstitial edema	-	-	++	-
Tubular dilation	-	-	++	-
Hyaline cast	-	+	+++	+
Medullary congestion	-	+	++	+

and subtle histological alterations were seen in the reperfusion + garlic group (Figure 5). There was no considerable pathologic alteration in the other groups (Table 3). Apoptotic renal epithelial cells and nuclear changes consisting of karyolysis, picnosis, and karyorhexia were also notified in the reperfusion group.

## DISCUSSION

The results of our study showed that pretreatment with garlic juice had the possible preventive effects against renal reperfusion injury of the kidney, as documented by functional parameters and histological examination. In our study, animals with reperfusion injury showed nephrotoxicity that was characterized by a decline in kidney function, increase of serum urea and creatinine, and decrease of urine creatinine, sodium, and potassium. These changes match to extensive histopathological damages such as cellular vacuolation, apoptosis, interstitial edema, tubular necrosis, and glomerular changes. However, with garlic juice pretreatment in the reperfusion group, functional and histopathological damages were reversed. It is probable that interstitial edema after

renal reperfusion results in increase of the ratio of kidney weight to body weight, and pretreatment with garlic juice improves edema.

Several investigations have demonstrated that a number of drugs, antioxidant enzymes, organic antioxidants, or agents that inhibit production of oxygen-free radicals, decrease the severity of reperfusion injury. Using agents such as deferoxamine, mannitol, tempol, uric acid, N-acetylcysteine, and plant extracts could protect against renal reperfusion injury.<sup>1</sup> In one study, renal damage was induced by intraperitoneal injection of 0.5 mg/kg of mercury chloride, and the rats were given garlic for 15 successive days prior to the injection. On the next 15 successive days simultaneously with the injection showed significant improvement of elevated serum urea and creatinine.<sup>23</sup>

Garlic and its various components are postulated to have an important cytoprotective role in the setting of reperfusion injury through their antioxidant and anti-inflammatory properties.<sup>16</sup> In kidney transplant recipients, results indicated that although serum creatinine and urea increased, but the patients who took one clove of garlic (1 g) by chewing or

swallowing for two months, serum levels of urea and creatinine did not increase.<sup>24</sup> S-allylcysteine, a water-soluble nontoxic garlic compound, has antioxidant properties both in vivo and in vitro. In an experiment that the effect of S-allylcysteine on renal injury and oxidative stress induced by ischemia and reperfusion was studied, treatment with S-allylcysteine was able to ameliorate the increase in serum urea and creatinine and to decrease the histopathological damage.<sup>25</sup> S-allylmercaptocysteine is another water soluble organosulfur compounds found in garlic extract. The protective effect of S-allylmercaptocysteine on gentamicin-induced nephrotoxicity was associated with decrease in serum urea and increase in creatinine clearance.<sup>26</sup>

Sodium nitrite, a food color fixative and preservative, contributes to carcinogenesis. In one study in response to sodium nitrite treatment, urea and creatinine were increased in the serum, suggesting an impairment of kidney function. These effects could also be attributed to the changes in the threshold of tubular reabsorption renal blood flow and glomerular filtration rate. Garlic oil showed a clear improvement in kidney functions, perhaps due to the antioxidant properties of garlic in scavenging free radicals leading to reduced levels of lipid peroxidation.<sup>27</sup>

It has also been shown that aqueous garlic extract (1 mL/kg, intraperitoneal, corresponding to 500 mg/kg), 15 minutes prior to ischemia and immediately before reperfusion treatment decreases ischemia reperfusion-induced injury in the rat's kidney.<sup>28</sup> The results of our study confirm that garlic juice pretreatment (24 hours and immediately before surgical procedure) protects the kidney against reperfusion injury.

## CONCLUSIONS

The results of this study showed that garlic juice pretreatment significantly protected renal reperfusion injury in rats. Administration of garlic juice attenuates the changes in markers of renal reperfusion injury.

## ACKNOWLEDGEMENTS

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## CONFLICT OF INTEREST

None declared.

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