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**Original Research Article**

# Prevention of injury by resveratrol in a rat model of adenine-induced chronic kidney disease

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## Abstract

***Purpose:*** *To investigate the preventive effect of resveratrol against renal pathological changes in a rat model of chronic kidney disease (CKD).*
***Methods:*** *CKD was induced by daily intragastric administration of adenine (200 mg/kg) for 1 month. The effect of 10, 15, and 20 mg/kg doses of resveratrol on the levels of parathyroid hormone, phosphorous, and fibroblast growth factor-23 (FGF-23) in rat urine samples after 2 months of adenine administration were analyzed using an auto-analyzer.*
***Results:*** *Resveratrol treatment significantly inhibited the adenine-mediated increase in serum parathyroid hormone, phosphorous and FGF-23 levels ($p < 0.002$). In rats treated with 10, 15 and 20 mg/kg doses of resveratrol after adenine, urine protein/creatinine ratio was reduced to 5, 675.6 ± 2453.7, 4, 789.8 ± 1,534.9, and 1, 965 ± 576.8 mg/g, respectively. In the untreated and normal control groups, the respective values were 7, 004 ± 1, 653.3 and 1, 627.5 ± 568.7 mg/g. Treatment with resveratrol after administration of adenine inhibited increases in creatinine, blood urea nitrogen, and uric acid levels in a dose-dependent manner ($p < 0.002$). Resveratrol treatment also inhibited adenine-mediated increases in monocytes and inflammatory cells. Furthermore, resveratrol prevented renal tubule swelling and expansion induced by adenine administration.*
***Conclusion:*** *Resveratrol treatment prevent the renal pathological changes induced by adenine administration in a rat model of CKD by inhibiting FGF-23, parathyroid hormone, and phosphate. Thus, resveratrol may be of therapeutic importance for the treatment of CKD.*

***Keyword:*** *Parathyroid hormone, Phosphate, Creatinine, Monocytes, Chronic kidney disease, Fibroblast growth factor-23*

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## INTRODUCTION

Chronic kidney disease (CKD) is associated with inflammation and hemodynamic changes that result in scarring of the renal tubules and interstitial spaces, and ultimately, the development of end-stage renal disease [1]. CKD is frequently diagnosed and currently represents the leading cause of death among patients with diabetes and hypertension [1].

Studies have shown that a prolonged increase in the serum levels of proteinuria and fibroblast growth factor-23 (FGF-23) is the main cause of end-stage renal disease [2,3]. Production of FGF-23 by bone catalyzes the excretion of inorganic phosphate from the kidneys via the

urine [4]. FGF-23 also inhibits the generation of vitamin D and maintains the balance between calcium and inorganic phosphate [4]. High levels of inorganic phosphate, parathyroid hormone, and FGF-23 in the serum promote the development of CKD [5]. Another factor that contributes to the development of kidney and heart disease is the presence of high levels of protein in urine [6]. Increased proteinuria interferes with the normal functioning of renal tubules and its suppression may be of therapeutic significance with respect to restoring normal kidney function. Therefore, reducing high levels of proteinuria should be a target for improving renal tubule function, and ultimately, treatment of CKD [6].

Trans-3, 5, 4'-trihydroxystilbene, commonly known as resveratrol, is a polyphenolic compound found in grapes, mulberries, and peanuts. Resveratrol exhibits a wide range of biological activities such as anti-fungal, anti-inflammatory, anti-mutagenic, and anti-cancer effects [7]. Resveratrol confers potent anti-inflammatory effects by inhibiting cellular inflammatory processes [8], and also inhibits the functions of chondrocytes by targeting the expression of interleukin-1β and inhibiting the generation of reactive oxygen species [9]. Resveratrol treatment also inhibits the production of inducible nitric oxide synthase by human epithelial cells [10]. In addition, it prevents cartilage degradation in animal models of arthritis [11]. This study examined the utility of resveratrol for treating CKD by suppressing the expression of inorganic phosphate, parathyroid hormone, and FGF-23 in a rat model.

## METHODS

### Chemicals and reagents

The adenine and resveratrol used in this study were obtained from Sigma-Aldrich (St. Louis, MO, USA).

### Animal study protocols

Twenty-five male Sprague-Dawley rats, each weighing about 200 g, were obtained from the Experimental Animal Center of Hebei Medical University [animal license: SCXK (Hebei 2015-2-034)]. The animal studies were approved by department of nephrology, Heping Hospital affiliated to Changzhi Medical College, Changzhi, Shanxi, China (Approval No: RXT52323). The rats were acclimatized to the laboratory conditions for 1 week before beginning the experiment, and were housed under a 12 h/12 h light/dark cycle at a constant temperature of 25 ± 1 °C and humidity in the range of 50 – 60 %. All of the animals had free access to food and water. The experimental protocols used were in accordance with the international suggestions for the Care and Use of Laboratory Animals (NIH publication no. 85-23, revised 1985) [12]. The animals were randomly assigned to five groups of five animals each. The rats in four of the groups (model group and three treatment groups) were administered adenine daily (200 mg/kg intragastrically) for 1 month. The three treatment groups were given 10, 15, and 20 mg/kg doses of resveratrol daily for 2 months, and the model group received normal saline.

Resveratrol was delivered through the intragastric route, and rats in the normal group received an equal volume of normal saline alone during the treatment period. Completion of treatment was followed by animal sacrifice, with halothane used to extract the kidney and collect the blood samples. At 24 h before sacrifice, urine was collected from each rat for analysis according to a previously reported protocol [13].

### Analysis of kidney damage

The levels of creatinine and blood urea nitrogen (BUN) in the blood and urine samples were determined using an automatic biochemistry analyzer (Hitachi 7600-020/7170A; Hitachi High-Technologies Corp., Tokyo, Japan). Briefly, the blood and urine samples collected from the rats were subjected to centrifugation for 15 min at 1, 120 × g. The levels of creatinine, BUN, parathyroid hormone, phosphorous, and uric acid in the urine samples, as well as the protein/creatinine ratio, were analyzed using the biochemistry analyzer [13].

### Analysis of pathological changes in renal tubules

The extracted kidneys were washed with phosphate-buffered saline (PBS) and fixed with buffered formalin (10 %), after which they were subjected to paraffin embedding, as previously reported [13]. The paraffin-embedded samples were cut into thin (2 µm) sections and boiled in xylene solution. The sections were stained with hematoxylin and eosin to analyze changes in renal tubule morphology under a magnification of 400×. Three regions of each section were examined randomly.

### Analysis of serum fibroblast growth factor-23

The levels of FGF-23 in rat serum samples were analyzed using an FGF-23 enzyme-linked immunosorbent assay (ELISA) kit (Immutopics,

San Clemente, CA, USA) according to the manufacturer's protocol. Briefly, the serum samples were subjected to centrifugation for 10 min at 1,500 rpm and subsequently stored at -20 °C for ELISA analysis. The serum samples were subjected to culture for 24 h after being added to 96-well microplates pre-coated with mouse monoclonal FGF-23 antibodies. After culture, the plates were washed and treated with horseradish peroxidase-conjugated polyclonal FGF-23 secondary antibodies. Then the plates were washed again and treated with $H_2O_2$ and tetramethylbenzidine followed by measurement of absorbance at 450 nm.

### Statistical analysis

The data are presented as means ± SD for each rat group. Analysis of the statistical differences between both groups of rats was done using unpaired Student's *t*-tests. Differences among all groups were analyzed by one-way analysis of variance with Bonferroni correction applied. All of the data were analyzed using SPSS software (ver. 12.0; SPSS, Inc., Chicago, IL, USA). $P < 0.05$ was taken as statistically significant.

## RESULTS

### Effects of resveratrol treatment on adenine-mediated upregulation of FGF23, inorganic phosphate, and parathyroid hormone in rat serum

Adenine administration markedly increased the levels of FGF-23, inorganic phosphate, and parathyroid hormone in rat serum compared to those of the control group ($p < 0.005$). However, treatment with resveratrol after adenine administration significantly reduced the increases in serum FGF-23, inorganic phosphate, and parathyroid hormone relative to the model control group (Figure 1). The serum levels of FGF-23 and parathyroid hormone were very similar between the normal and resveratrol-treated (20 mg/kg) rats.

### Effects of resveratrol treatment on adenine-mediated increase in urine protein/creatinine ratio

In rats administered adenine, the urine protein/creatinine ratio was markedly increased compared to that of the normal group. Treatment with resveratrol after adenine significantly attenuated this increase in the protein/creatinine ratio ($p < 0.001$). In the rats treated with 10, 15, and 20 mg/kg doses of resveratrol after adenine administration, the urine protein/creatinine ratio was reduced significantly, to 5, 675.6 ± 2, 453.7, 4, 789.8 ± 1, 534.9 and 1, 965 ± 576.8 mg/g, respectively, compared to values in the model control group (7, 004 ± 1, 653.3). The urine protein/creatinine ratio of the normal control group was 1, 627.5 ± 568.7 mg/g (Figure 2). The increase in uric acid level caused by adenine administration was also significantly reduced by treatment with resveratrol in all three dose groups ($p < 0.002$).

### Resveratrol treatment lowered the adenine-mediated increase in serum creatinine and BUN levels

In rats administered adenine, the serum levels of creatinine and BUN were markedly increased compared to those of the normal group ($p < 0.002$). Treatment with resveratrol after administration of adenine attenuated the increases in creatinine and BUN levels in a dose-dependent manner (Figure 3).

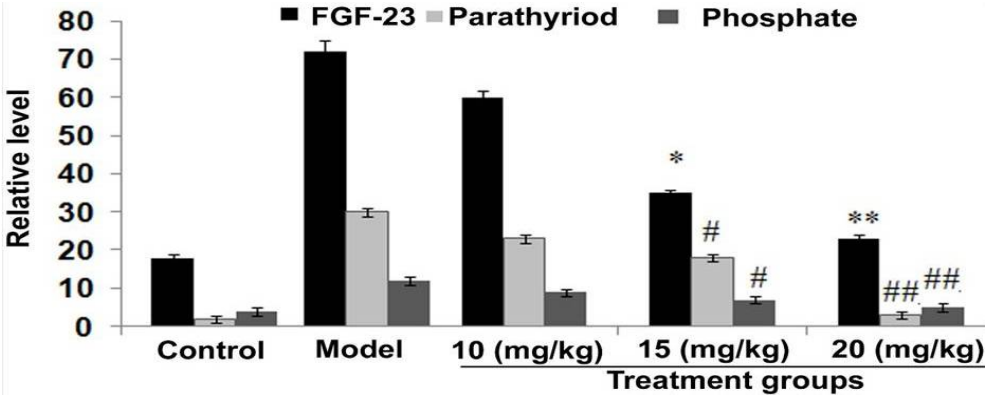


**Figure 1:** Effects of resveratrol on adenine-induced increases in fibroblast growth factor-23 (FGF-23), inorganic phosphate, and parathyroid hormone levels. Rats in the treatment groups were given 10, 15, and 20 mg/kg doses of resveratrol after adenine administration, whereas the normal rats only received saline. The control group was given normal saline after adenine administration; *$p < 0.05$, **$p < 0.01$, #$p < 0.05$, and ##$p < 0.01$ compared to the model control group

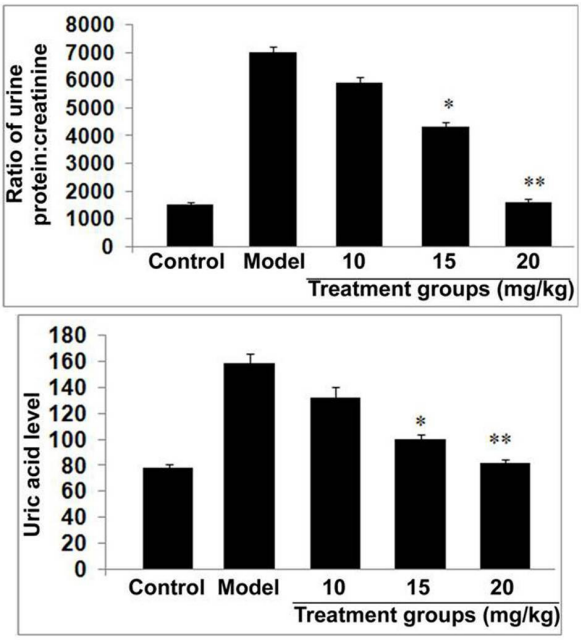


**Figure 2:** Effects of resveratrol treatment on the adenine-mediated increase in the urine protein/creatinine ratio. Rats administered adenine were treated with 10, 15, and 20 mg/kg doses of resveratrol or normal saline (control). The urine of the rats was collected over a 24 h period to determine the levels of protein and creatinine; *$p < 0.05$ and **$p < 0.01$ compared to the untreated control group

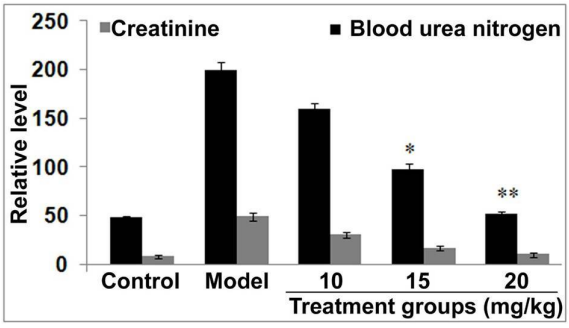


**Figure 3:** Effects of resveratrol treatment on levels of creatinine and blood urea nitrogen (BUN). Rats administered adenine were treated with 10, 15, and 20 mg/kg doses of resveratrol or normal saline (control). The serum levels of creatinine (µmol/L) and BUN (mmol/L) were analyzed; *$p < 0.05$, **$p < 0.01$, #$p < 0.05$ and ##$p < 0.01$ compared to the untreated control group

**Resveratrol treatment prevents adenine-induced pathological changes in rat kidney**

Administration of adenine caused changes in the renal tubules in the kidneys of the rats. Examination of the renal tubules of rats administered adenine revealed marked swelling and expansion ($p < 0.005$). The tubules in the adenine groups had higher levels of dihydroxyadenine than normal control rats, and the renal tissues also had marked increases in monocytes and inflammatory cells ($p < 0.002$; Figure 4). Resveratrol treatment inhibited the adenine-mediated increase in monocytes and inflammatory cells and prevented renal tubule swelling and expansion (Figure 4).

## DISCUSSION

Acute kidney injury is encountered frequently during clinical practice and affects the survival of patients. Injury to the renal tubules leads to acute renal failure in more than 80 % of patients [14,15]. This study demonstrated curative effects of resveratrol on the renal pathological changes occurring in a rat model of CKD, in which the animals were administered adenine intragastrically.

Levels of phosphorus and calcium in the kidneys are regulated and maintained by various hormones such as parathyroid hormone, calcitriol, and FGF-23 [16]. Upregulation of phosphorus, FGF-23, and parathyroid hormone is associated with worsening kidney disease and a higher likelihood of death [17,18]. This study demonstrated a marked decrease in adenine-induced increases in serum phosphorus, FGF-23, and parathyroid hormone levels in rats, suggesting the preventative effects of resveratrol against adenine-mediated kidney disorders. The increased urine protein/creatinine ratio caused by adenine administration was also attenuated by treatment with resveratrol. Furthermore, our study demonstrated that rats administered adenine had higher serum levels of creatinine, BUN, and uric acid. Treatment of rats with resveratrol after administration of adenine inhibited the increases in creatinine, BUN, and uric acid levels in a dose-dependent manner.

The results of this study showed that adenine administration caused changes in the renal tubules in the kidneys of rats; there was considerable swelling and expansion of the renal tubules. The level of dihydroxyadenine in the renal tubules of rats administered adenine was markedly higher than that of the normal control rats, and the renal tissues also had markedly increased levels of monocytes and inflammatory cells. However, resveratrol treatment inhibited the adenine-mediated increase in monocytes and inflammatory cells and prevented renal tubule swelling and expansion.

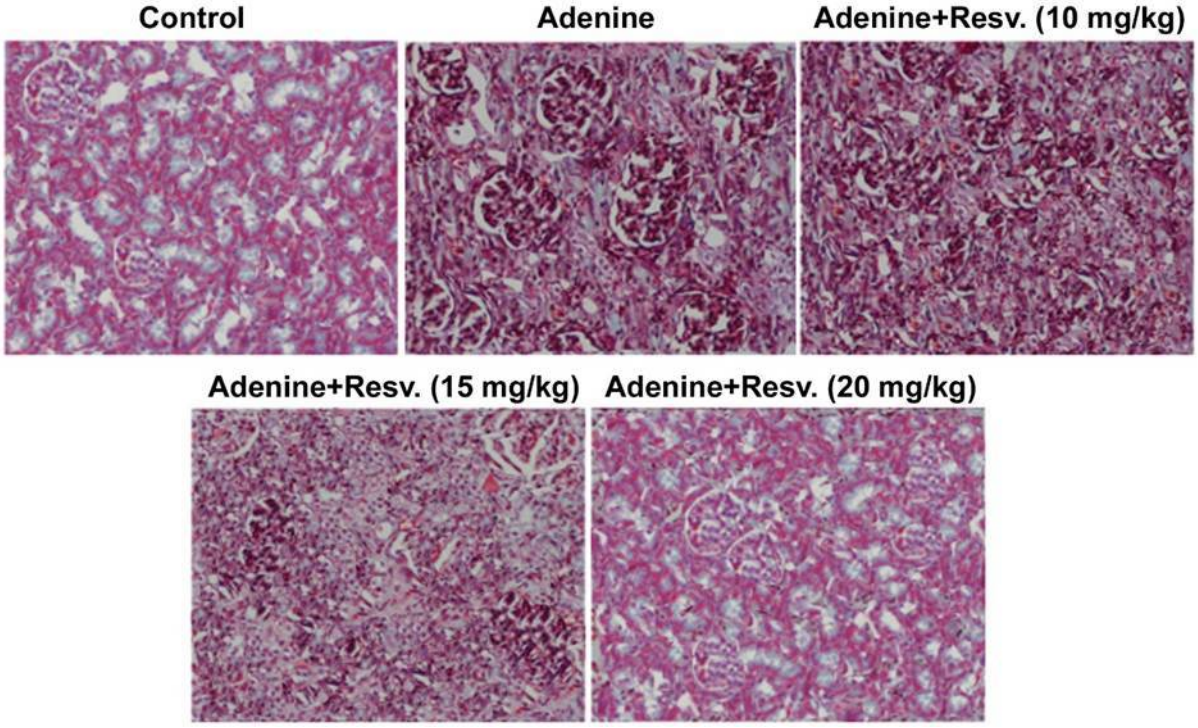


**Figure 4:** Resveratrol treatment prevented adenine-induced pathological changes in the rat kidney. Rats administered adenine were treated with resveratrol at 10, 15, and 20 mg/kg doses or normal saline (control). Renal tissues were examined for pathological changes after staining with hematoxylin and resveratrol.

## CONCLUSION

Resveratrol treatment prevents renal pathological changes induced by adenine administration in a CKD rat model by inhibiting FGF-23, parathyroid hormone, and phosphate. Thus, resveratrol may have therapeutic importance for the treatment of CKD.

## DECLARATIONS

### *Acknowledgement*

The authors are thankful to the Department of Nephrology, Affiliated Heping Hospital of Changzhi Medical College, Shanxi, China, for financial support.

### *Conflict of Interest*

No conflict of interest associated with this work.

### *Contribution of Authors*

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.



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