

The Effects of Angiotensin-Converting-Enzyme Inhibitor (Captopril) on Gentamicin Nephrotoxicity in Rats.

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ABSTRACT

This study was designed to investigate the effects of captopril on gentamicin nephrotoxicity. Six groups of male Wister rats "15 rats each" were used. Group "C", was given 1ml of normal saline, group "G25" was injected with a single dose of GM (25mg/Kg/day), group "G50" was injected with a single dose of GM (50mg/Kg/day), group "Cp2" was given a single oral dose of captopril (2mg/day), group "Cp2+G25" was injected with a single dose of GM (25mg/Kg/day) and a single oral dose of captopril (2mg/day), group "Cp2+G50" was injected with a single dose of GM (50mg/Kg/day) and a single oral dose of captopril (2mg/day). Treatment continued daily for 15 days; analysis was performed on days 5, 10 and 15. Blood urea nitrogen and serum creatinine levels were significantly elevated on the 5th day and continued to increase throughout the duration of the experiment; there was a gradual decrease in the creatinine clearance level, starting from the 5th day of treatment, which was related to the duration and dosage used in the GM treated rats. The administration of captopril progressively reduced the functional deteriorations induced by GM. It is concluded that captopril reduces GM-induced renal damage, suggesting protective effect of captopril against GM nephrotoxicity.

INTRODUCTION

Nephrotoxicity refers to dangerous kidney problems that develop when toxins accumulate in the kidneys. Toxins and unneeded fluids are usually excreted through urine, but when they start reaching excessive levels, they eventually lead to nephrotoxicity and cause a variety of other symptoms of kidney trouble [1]. Aminoglycosides are a group of bactericidal drugs sharing chemical, antimicrobial, pharmacological and toxic characteristics. They are one of the commonest causes of drug-induced nephrotoxicity [2]. Aminoglycosides are nephrotoxic because a small amount of the administered dose is retained and accumulate in the endosomal and lysosomal vacuoles and the Golgi apparatus of the proximal convoluted tubules [3]. In humans, these changes are accompanied by signs of tubular dysfunctions and may be followed by the development of renal failure [4].

Gentamicin is a broad-spectrum aminoglycoside antibiotic, very effective in treating gram-negative bacterial infections in both humans and animals, especially of the urinary tract [5]. About 30% of patients treated with GM for more than seven days develop signs of nephrotoxicity [6]. The nephrotoxicity of GM has been shown to involve an inflammatory process in humans [7] and experimental animals [8, 9]. It has been demonstrated that GM nephrotoxicity is a complicated phenomenon characterized by an increase in serum creatinine and urea levels and severe proximal tubular necrosis, followed by deterioration and renal failure [10].

Angiotensin-converting-enzyme (ACE) inhibitors are pharmaceutical drugs used primarily for the treatment of hypertension and congestive heart failure; they block the conversion of angiotensin I into angiotensin II. Captopril is an ACE inhibitor used for the treatment of hypertension [11], congestive heart failure [12] and diabetic renal failure

[13]. It also has hepatoprotective activities against cisplatin-induced cytotoxicity [14]. The present study was undertaken to investigate the potential protective effects of captopril on the manifestation of renal function changes induced by GM.

ABBREVIATIONS: ACE, Angiotensin-converting-enzyme; GM, Gentamicin; BUN, Blood urea nitrogen.

MATERIALS AND METHODS

Animals

Ninety young adult male Wister rats weighing (250 - 300g) each, were in good health and kept under suitable environmental conditions of $24\pm 2^{\circ}\text{C}$. They had free access to laboratory chow and water, and were exposed to a 12 hour/day light program.

Experimental design

The animals were randomly divided into six groups with 15 rats in each: the first "C" control group was given one ml of normal saline; the second "G 25" group was injected with a single dose of GM (25mg/Kg/day); the third "G 50" group was injected with a single dose of GM (50mg/Kg/day); the fourth "Cp2" group was given a single oral dose of captopril (2mg/day); the fifth "Cp2+G25" group was injected with a single dose of GM (25mg/Kg/day) and at the same time given a single oral dose of captopril (2mg/day); and the sixth "Cp2+G50" group was injected with a single dose of GM (50mg/Kg/day) and at the same time given a single oral dose of captopril (2mg/day). Animals were transferred to a metabolic cage 24 hours before the euthanasia to collect the urine.

The animals were sacrificed with light ether anaesthesia on days 5, 10 or 15. After the sacrifice, the abdominal cavity was opened; blood samples were obtained by cardiac puncher. The impairment of renal function was investigated by determining blood urea nitrogen "BUN" levels, serum creatinine concentrations and creatinine clearance [15]. The experimental animal protocol was conducted in compliance with humane animal care standards outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals, the ethic number of Al-Mustansiriah Univ. 000 (M) 01.

Statistical Analysis

The differences between the experimental and control groups were statistically evaluated using Student t-test. The results were expressed as mean \pm standard deviation. P values less than 0.05 were considered to be significant. One way analysis of variance (ANOVA) was used to test the significant difference between the treated groups.

RESULTS

The 5th day of treatment

There were no significant changes in the control "C" group during the whole period of the experiment. Rats injected with GM in the group "G25" presented a significant increase ($P < 0.05$) in BUN (35.74 ± 2.2 mg/100ml) compared to control (28.8 ± 1.2 mg/100ml), as well as a significant decrease ($P < 0.05$) in creatinine clearance (0.05971 ± 0.02 ml/min) compared to control (1.01386 ± 0.03 ml/min). However, no significant differences were found in the serum creatinine level ($P > 0.05$).

Group "G50" rats showed a significant increase ($P < 0.05$) in serum creatinine level (0.72 ± 0.04 mg/100ml) compared to control (0.6 ± 0.07 mg/100ml); there was also a significant increase ($P < 0.05$) in BUN (39.28 ± 0.7 mg/100ml) and a significant decrease ($P < 0.05$) in creatinine clearance (0.46071 ± 0.1 ml/min), compared to control.

Group "CP2" rats did not show any significant changes in BUN, serum creatinine levels and creatinine clearance.

Group "CP2 + G25" rats showed a significant increase ($P < 0.05$) in creatinine clearance (0.794 ± 0.1 ml/min) compared to the group "G25" rats (0.05971 ± 0.02 ml/min). No significant changes were observed in BUN and serum creatinine levels compared to the "G25" group.

In the "CP2 + G50" group, there was a significant decrease ($P < 0.05$) in serum creatinine levels (0.6 ± 0.07 mg/100ml) compared to the "G50" group (0.72 ± 0.04 mg/100ml); there was also a significant decrease ($P < 0.05$) in BUN (36.04 ± 1.1 mg/100ml) compared to the "G50" group (39.28 ± 0.7 mg/100ml). Creatinine clearance

showed a significant increase ($P < 0.05$) (0.737 ± 0.08 ml/min) compared to the “G50” group (0.46071 ± 0.1 ml/min).

The 10th day of treatment

Group “G25” rats showed a significant increase ($P < 0.05$) in the serum creatinine level (0.7 ± 0.07 mg/100ml) compared to control rats (0.56 ± 0.1 mg/100ml). BUN was also elevated significantly ($P < 0.05$) (38.37 ± 1.05 mg/100ml) compared to control (29.08 ± 0.9 mg/100ml). Creatinine clearance, however, declined significantly ($P < 0.05$) (0.503456 ± 0.1 ml/min) compared to control (1.013343 ± 0.05 ml/min).

The “G50” group also showed a significant increase ($P < 0.05$) in serum creatinine levels (0.8 ± 0.07 mg/100ml) compared to control (0.56 ± 0.1 mg/100ml). At the same time, a significant increase ($P < 0.05$) in BUN was observed (42.22 ± 7.1 mg/100ml) compared to control rats (29.08 ± 0.9 mg/100ml). A significant decline ($P < 0.05$) was noticed in creatinine clearance (0.312776 ± 0.05 ml/min) in relation to the control rats (1.013343 ± 0.05 ml/min).

Group “CP2” rats also did not show any significant changes in the BUN, serum creatinine levels and creatinine clearance after 10 days of treatment.

There was a significant increase ($P < 0.05$) in BUN in the “CP2 + G25” group (34.66 ± 1.09 mg/100ml) compared to “G25” rats (38.38 ± 1.05 mg/100ml), as well as a significant rise ($P < 0.05$) in creatinine clearance (0.809285 ± 0.1 ml/min) in relation to the “G25” rats (0.503456 ± 0.1 ml/min). However, no significant changes ($P > 0.05$) were observed in the serum creatinine levels of the “CP2 + G25” group in comparison to “G25” rats. “CP2 + G50” rats showed a significant decline ($P < 0.05$) in serum creatinine concentrations (0.66 ± 0.05 mg/100ml) compared to the “G50” rats (0.8 ± 0.07 mg/100ml); there was a significant increase ($P < 0.05$) in creatinine clearance (0.726983 ± 0.07 ml/min) in relation to “G50” rats (0.312776 ± 0.05 ml/min). No significant changes ($P > 0.05$) were observed in the BUN levels of “CP2 + G50” rats compared to “G50” rats.

The 15th day of treatment

Group “G25” rats showed a significant increase ($P < 0.05$) in serum creatinine levels (0.74 ± 0.05 mg/100ml) compared to control rats (0.64 ± 0.5 mg/100ml). BUN was also increased significantly ($P < 0.05$) (39.18 ± 1.2 mg/100ml) compared to control (29.08 ± 0.9 mg/100ml). However, creatinine clearance declined significantly ($P < 0.05$) (0.315889 ± 0.1 ml/min) in relation to control rats (1.01494 ± 0.05 ml/min).

Group “G50” rats also showed a significant increase ($P < 0.05$) in serum creatinine levels (0.84 ± 0.05 mg/100ml) and BUN (48.64 ± 2.9 mg/100ml), in comparison to control rats. A significant reduction ($P < 0.05$) in creatinine clearance was observed in this group (0.190116 ± 0.04 ml/min) compared to control rats (1.01494 ± 0.07 ml/min).

No significant changes in the BUN, serum creatinine level and creatinine clearance were observed in group “CP2” rats. The “CP2 + G25” group showed a significant decrease ($P < 0.05$) in serum creatinine concentrations (0.62 ± 0.08 mg/100ml) compared to “G25” rats (0.74 ± 0.05 mg/100ml), as well as a significant decrease ($P < 0.05$) in BUN (34.44 ± 1.4 mg/100ml) compared to the “G25” group (39.18 ± 1.2 mg/100ml). This group also showed a significant increase ($P < 0.05$) in creatinine clearance (0.818079 ± 0.1 ml/min) in relation to the “G25” rats (0.315889 ± 0.1 ml/min). Serum creatinine was significantly decreased ($P \leq 0.05$) in “CP2 + G50” rats (0.66 ± 0.05 mg/100ml) compared to “G50” rats (0.84 ± 0.05 mg/100ml); BUN was also significantly decreased ($P < 0.05$) (35.66 ± 2.5 mg/100ml) in relation to the “G50” rats (48.64 ± 2.9 mg/100ml). However, creatinine clearance was significantly improved ($P < 0.05$) (0.801469 ± 0.1 ml/min) compared to the “G50” group (0.190116 ± 0.04 ml/min).

Table 1: The values of serum creatinine, BUN and creatinine clearance at the 5th day of the experiment.

Groups	Serum creatinine	BUN	Creatinine clearance
C	0.6±0.07	28.8±1.2	1.01386±0.03
G25	0.66±0.05*	35.74±2.2*	0.5971±0.02#
G50	0.72±0.04*	39.28±0.7*	0.4607±0.01*
CP2	0.58±0.04#	30.06±0.2#	1.00171±0.05#
CP2+G25	0.62±0.08#	35.16±1.9#	0.794±0.01**
CP2+G50	0.6±0.07\$	36.04±1.1\$	0.737±0.08\$

Values are presented as mean + SEM.

*: Significantly ($P < 0.05$) different from its control. #: Not significantly ($P > 0.05$) different from its control. **: Significantly ($P < 0.05$) different from G25 group. \$: Significantly ($P < 0.05$) different from G50 group.

Table 2: The values of serum creatinine, BUN and creatinine clearance at the 10th day of the experiment.

Group	Serum creatinine	BUN	Creatinine clearance
C	0.56±0.01	29.8±0.9	1.01334±0.05
G25	0.7±0.07*	38.38±1.05*	0.503456±0.01*
G50	0.8±0.07*	42.22±7.1*	0.312776±0.05*
CP2	0.58±0.04#	30.1±0.5#	1.0165±0.01#
CP2+G25	0.6±0.07 ^υ	34.66±1.09**	0.809285±0.01**
CP2+G50	0.66±0.05\$	35.24±1.7 ^α	0.726983±0.07\$

Values are presented as mean + SEM.

*: Significantly (P<0.05) different from its control. #: Not significantly (P>0.05) different from its control. **: Significantly (P<0.05) different from G25 group. ^υ: Not significantly (P>0.05) different from G25 group. \$: Significantly (P<0.05) different from G50 group. ^α: Not significantly (P>0.05) different from G50 group.

Table 3: The values of serum creatinine, BUN and creatinine clearance at the 15th day of the experiment.

Group	Serum creatinine	BUN	Creatinine clearance
C	0.64±0.05	29.65±0.9	1.01494±0.07
G25	0.74±0.05*	39.18±1.2*	0.315889±0.01*
G50	0.84±0.05*	48.64±2.9*	0.190116±0.04*
CP2	0.6±0.07#	29.82±0.4#	1.00414±0.06#
CP2+G25	0.62±0.08**	34.44±1.4**	0.818079±0.01**
CP2+G50	0.66±0.05\$	35.66±2.5\$	0.801469±0.01\$

Values are presented as mean + SEM.

*: Significantly (P<0.05) different from its control. #: Not significantly (P>0.05) different from its control. **: Significantly (P<0.05) different from G25 group. \$: Significantly (P<0.05) different from G50 group.

DISCUSSION

The present investigation demonstrated that captopril has a protective effect against the nephrotoxicity of GM. Treatment with 25 mg/Kg/day or 50mg/Kg/day of GM caused functional renal injury. There was gradual elevation in BUN from the 5th day towards the end of the experiment on the 15th day (Fig 1). This elevation was proportional to the dosages used and was consistent with the BUN elevations observed in rats treated with GM and amikacin [16].

The present study also demonstrated an increase in serum creatinine concentrations. This elevation started on the 10th day of treatment with 25mg/Kg/day of GM and on the 5th day in the animals treated with 50mg/Kg/day (Fig 2). These observations also related to the dosage used, as well as treatment duration. Dellinger *et al.*, [17] found that rats treated with 6 to 50 mg/kg/day of GM for 10 days produced tubular necrosis scores that increased linearly with increasing doses of the antibiotic. Moreover, these observed indices of renal injury were associated with significantly higher BUN and serum creatinine concentrations.

The present study demonstrated a gradual reduction in creatinine clearance from the 5th day of treatment. This decline was greater with increased duration and dose (Fig 3). This is consistent with the results of Morin *et al.*, [18], who observed a similar gradual decrease in creatinine clearance starting from the 2nd day of treatment with 50 mg/kg/ day GM in rats and continuing for 15 days. A gradual decline in creatinine clearance was also demonstrated in rats injected with 40 mg/kg/ day GM for 10 days [19]. Creatinine and BUN levels were significantly higher (P < 0.05) and creatinine clearance significantly lower (P < 0.05) in female Sprague-Dawley rats treated with a single daily injection of GM (20 and 40 mg/kg) for 3 and 7 days [20]. Administration of GM at a dose of 100 mg/kg/day for 8 consecutive days induced a significant elevation in the biochemical parameters of kidney function such as BUN, serum creatinine and urea [21, 22]. Significant increases in the levels of serum creatinine, urea and urea nitrogen, as well as urinary excretion of protein were observed in albino rats injected with 80 mg/kg/day GM for ten days [23]. Elevation in BUN and serum creatinine concentrations, in addition to a reduction of sodium and potassium levels associated with renal histopathological changes, were also detected in rats treated with 100mg/Kg GM for 8 days [24]. Treatment of male Wistar rats with 40 mg/kg GM, twice a day, for 9 days elicited increases in serum creatinine and BUN levels, which were associated with tubular interstitial changes in the cortex [25].

The concomitant administration of captopril and GM in the “CP2 + G25” and “CP2 + G50” groups showed that captopril gradually improved the deteriorations in renal functions induced by GM. The improvement occurred on the 5th day and continued throughout the duration of the treatment, suggesting that captopril can reduce GM-induced nephrotoxicity. This is in support of previous studies on human patients; captopril significantly reduced the risk of renal failure progression in patients with diabetic nephropathy [26]. Patients with chronic renal failure treated with captopril for one year showed lower serum creatinine levels and higher glomerular filtration rate compared to the placebo groups, suggesting that captopril protected against chronic renal failure [13].

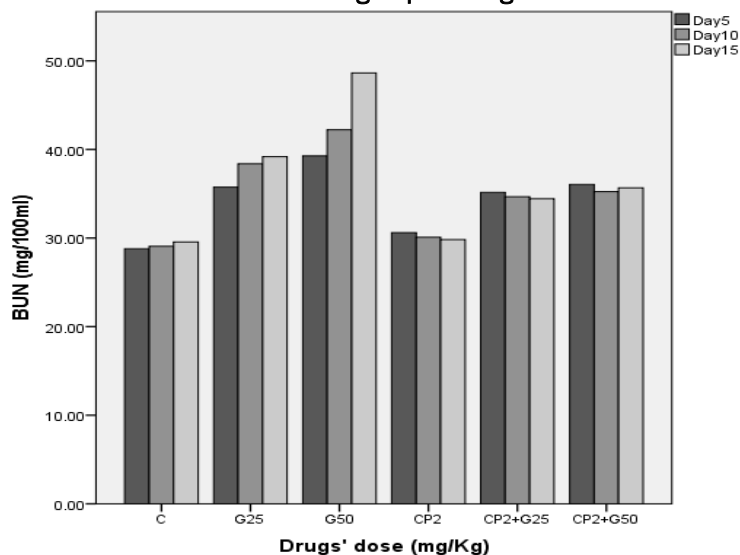
The mechanisms by which GM induce nephrotoxicity are not clear yet. Pedraza-Chaverri *et al.*, [27] suggested the generation of reactive oxygen species in the development of GM nephrotoxicity, causing deficiency in intrinsic antioxidant enzymes [28]. Administration of GM stimulates cortical lipid peroxidation and mitochondrial hydrogen peroxide production [29]. These abnormal productions by GM lead to cellular degeneration by protein denaturation, lipid peroxidation and DNA damage [30, 31].

The precise mechanism by which captopril reduces GM nephrotoxicity remains to be determined. The ability of captopril to reduce GM nephrotoxicity might be related to its anti-inflammatory effect. This anti-inflammatory effect has been linked to the activation of NF-KB-dependent pro-inflammatory factors [nuclear factor kappa beta] and demonstrated in experimental studies of increased inflammation process, such as arthritic rats [32], adriamycin nephropathy in mice [33] or spontaneously hypertensive rats [34].

Antioxidants play a major protective role against GM-induced nephrotoxicity; scavengers of reactive oxygen metabolites and iron chelators have shown to be protective in GM-induced acute renal failure [2]. The administration of antioxidants relieves GM-induced kidney damage [35, 36]. Kadkhodae *et al.*, [37] suggested the potential of antioxidant vitamins to protect against GM-induced nephrotoxicity. Captopril has been suggested to lower angiotensin II and, in turn, oxidant stress that may contribute to the blood pressure-lowering efficacy of captopril in spontaneously hypertensive rats [38]. The role of captopril as an antioxidant in the protection against GM-induced nephrotoxicity is still to be investigated.

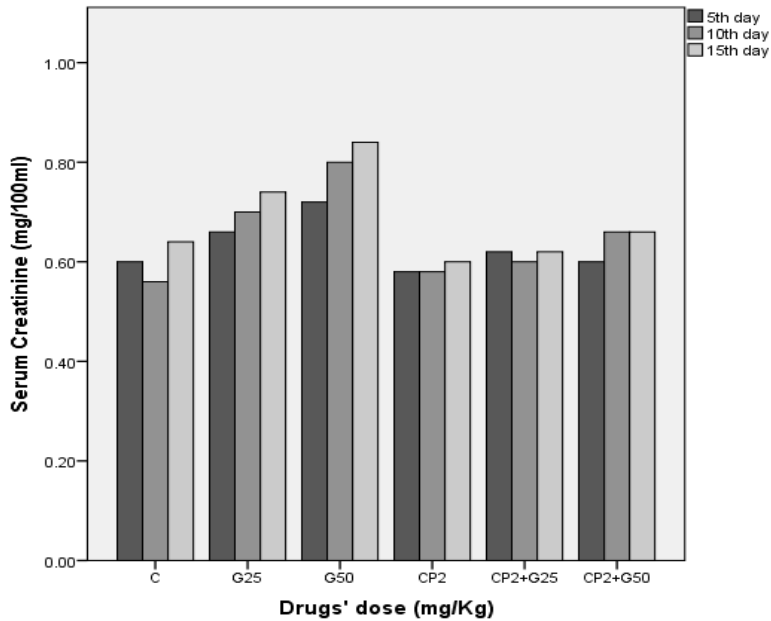
Captopril has been suggested to enhance GM excretion. A gradual increase in GM excretion was observed in the urine of rats treated with both captopril and GM. Moreover, this GM excretion was related to dosage and duration [39]. Morphological deterioration involving the renal tubules, which show patchy necrosis along with hyaline and granular casts in their lumina, was observed in rabbits treated with 60 and 150 mg/ Kg/day of GM for 20 days [40]. Histological and ultrastructural studies related to the effect of captopril on GM nephrotoxicity are in progress.

Figure 1: Levels of BUN in different groups throughout the duration of the experiments.



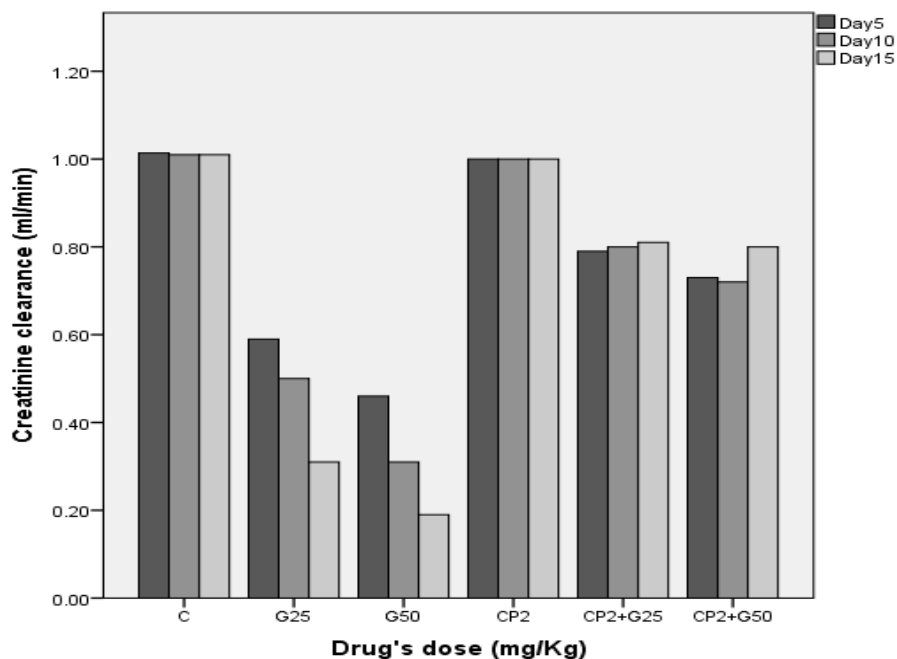
C: Control rats, given one ml of normal saline/day. G25: Rats injected with a single dose of GM (25mg/Kg/day). G50: Rats injected with a single dose of GM (50mg/Kg/day). CP2: Rats given a single oral dose of captopril (2mg/day). Cp2+G25: Rats injected with a single dose of GM (25mg/Kg/day) and a single oral dose of captopril (2mg/day). Cp2+G50: Rats injected with a single dose of GM (50mg/Kg/day) and a single oral dose of captopril (2mg/day).

Figure 2: Levels of serum creatinine in different groups throughout the duration of the experiment.



C: Control rats, given one ml of normal saline/day. G25: Rats injected with a single dose of GM (25mg/Kg/day). G50: Rats injected with a single dose of GM (50mg/Kg/day). CP2: Rats given a single oral dose of captopril (2mg/day). Cp2+G25: Rats injected with a single dose of GM (25mg/Kg/day) and a single oral dose of captopril (2mg/day). Cp2+G50: Rats injected with a single dose of GM (50mg/Kg/day) and a single oral dose of captopril (2mg/day).

Figure 3: Levels of creatinine clearance in different groups throughout the duration of the experiments.



C: Control rats, given one ml of normal saline/day. G25: Rats injected with a single dose of GM (25mg/Kg/day). G50: Rats injected with a single dose of GM (50mg/Kg/day). CP2: Rats given a single oral dose of captopril (2mg/day). Cp2+G25: Rats injected with a single dose of GM (25mg/Kg/day) and a single oral dose of captopril (2mg/day). Cp2+G50: Rats injected with a single dose of GM (50mg/Kg/day) and a single oral dose of captopril (2mg/day).

CONCLUSION

In conclusion, this study showed that serum creatinine and BUN concentrations were augmented, while creatinine clearance was decreased in GM-treated rats, indicating renal damage. Treatment with captopril reduced GM-induced renal damage, suggesting a protective effect of captopril against GM nephrotoxicity.

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