

Calcium Dobesilate for Prevention of Gentamicin-induced Nephrotoxicity in Rats

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Introduction. Calcium dobesilate is an antioxidant drug and this study is aimed to investigate the effects of calcium dobesilate on gentamicin-induced nephrotoxicity.

Material and Methods. This experimental study was conducted on 40 male Sprague-Dawley rats. The rats were randomly divided into the following 5 groups: control, sham, gentamicin (100 mg/kg/d, intraperitoneal), gentamicin and calcium dobesilate (50 mg/kg body weight), gentamicin and calcium dobesilate (100 mg/kg body weight). Treatment was provided once a day in a 7-day period. At the end of the 7th day, plasma and urine samples were taken and the concentrations of creatinine, urea nitrogen, sodium, potassium, and osmolarity were measured in both samples. The level of oxidative stress in the left kidney tissue samples was also assessed by measuring malondialdehyde and ferric reducing antioxidant power.

Results. Calcium dobesilate intake with both doses led to a significant decrease in the elevated concentration of creatinine, urea nitrogen, and fractional excretion of sodium by gentamicin, and an increase in creatinine clearance and absolute excretion of potassium as compared to the gentamicin group. Furthermore, calcium dobesilate decreased tissue malondialdehyde and increased tissue ferric reducing antioxidant power at both doses, in comparison to those in the gentamicin group.

Conclusions. Calcium dobesilate is capable of protecting rats against gentamicin-induced nephrotoxicity. This protective effect of calcium dobesilate is probably dependent on its antioxidant properties.

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INTRODUCTION

Gentamicin is usually used for the treatment of infections caused by gram-negative bacteria.^{1,2} Unfortunately, up to 30% of the patients that are treated with gentamicin for more than 7 days develop nephrotoxicity.^{3,4} There is not a full understanding of mechanisms that lead to gentamicin-induced nephrotoxicity, but it has been suggested that pathological mechanisms that are involved include increased generation

of reactive oxygen species (ROS), induction of apoptosis, necrosis, increased endothelin-1, and increased infiltration of monocytes-macrophages.⁵⁻⁹ Gentamicin leads to lipids peroxidation and damages to cellular and intracellular membranes.^{10,11} Also, gentamicin-induced nephrotoxicity is characterized by increased concentration of plasma creatinine and urea nitrogen, increased fractional excretion of sodium, decreased glomerular filtration rate (GFR), and urine osmolarity.¹² Several studies

have indicated that consumption of antioxidant compounds reduces the possibility of nephrotoxicity caused by gentamicin.¹³⁻¹⁵

Calcium dobesilate is one of the derivatives of cyclohexadienilic bisulphate, which decreases vascular permeability and is used for the treatment of diabetic retinopathy.^{16,17} In addition, it has been indicated that calcium dobesilate is a strong antioxidant.¹⁸ In vitro studies suggest that calcium dobesilate acts as a free radical scavenger, while these experiences have also been verified by animal studies as well.¹⁹ The objective of this study was to investigate the effects of calcium dobesilate in prevention of gentamicin-induced nephrotoxicity, hypothesizing that the strong antioxidant properties of calcium dobesilate can prevent oxidative stress and consequently creation of ROS induced by gentamicin.

MATERIALS AND METHODS

Methods

This experimental study involved 40 Sprague-Dawley rats with weights of 200 g to 300 g, bred in the Animal Center of Arak University of Medical Sciences. These animals were kept 12 hours in darkness and 12 hours in light at a temperature of $23 \pm 2^\circ\text{C}$ with free access to water and food. All the experiments were carried out by considering and implementing ethical codes defined by the Ministry of Health and Medical Education of Iran for performing tests on laboratory animals.

The animals were randomly divided into 5 groups ($n = 8$). The control group did not receive any solvent or drug during experiments and was on a usual diet. The sham group received 0.5 mL of normal saline on a daily basis for 7 days through intraperitoneal injection. The gentamicin group received 100 mg/kg of gentamicin (Alborz Co, Tehran, Iran) intraperitoneally and 0.5 mL of normal saline, as drug solvent, for 7 days. The protocol for the fourth group (D50) was similar to that of the gentamicin group except that this group also received 50 mg/kg of calcium dobesilate (Doxium, OM Laboratories, Geneva, Switzerland) every 24 hours, by performing gavage by 7 days. Finally, the last group (D100) received 100 mg/kg of calcium dobesilate per day by gavage for a 7-day period, as well as gentamicin. Calcium dobesilate was administered on a daily basis by gavage 1 hour after gentamicin.

When all the animals received their last dose of calcium dobesilate and gentamicin, they were put in separate metabolic cages for a 6-hour urine collection period. After collecting urine samples, using thiopental (50 mg/kg, intraperitoneal) the animals were anesthetized and blood samples were taken from their abdominal aorta. Then, the left kidneys were rapidly frozen and kept at -20°C in order to measure malondialdehyde resulted from peroxidation of lipids by reactive oxygen species and ferric reducing antioxidant power (FRAP). The concentrations of sodium, potassium, urea nitrogen, and creatinine as well as the osmolarity of urine and plasma samples were measured. Then, fractional and absolute excretions of sodium and potassium were calculated in order to examine the performance of the kidneys and creatinine clearance as an index of GFR.

Measurement of Malondialdehyde

As described in our previous reports,²⁰⁻²² in order to determine the level of malondialdehyde in kidney tissue, the Ohkawa method was employed.²³ After the kidney was removed and weighed, phosphate buffer was added to it with a ratio of 1 to 10 and it was homogenized using a homogenizer (Loughborough, UK). Afterwards, acetic acid (20%), thiobarbituric acid (0.8%), and sulfate deododecyl sodium (8.1%) were added to all the test tubes. Tubes containing the abovementioned tissue suspension were heated for 60 minutes at 95°C . After interaction with malondialdehyde, a pink complex was formed that was extracted by using N-butanol. Next, the amount of optical absorption was measured at a wave length of 523 nm using a Jane Way spectrophotometer. The results were expressed as nanomole per gram kidney weight (nmol/g KW).

Measurement of Ferric Reducing Antioxidant Power

Measurement of FRAP is one of the most common ways of assessing total antioxidant activity. This method is based on the capacity of tissue fluids for converting ferric ions into ferrous ion in the presence of tripyridyl-s-triazine. The Benzie and Strain method was used for measuring FRAP.²⁴ First, a fresh FRAP reagent was prepared. The temperature of this reagent, which contained the following constituents, was increased to 37°C : 2.5

mL of 10-mmol tripyridyl-s-triazine solution in 40 mmolar hydrochloric acid, 2.5 mL of ferric chloride, and 2.5 mL of 0.3-mol acetate buffer with a pH of 3. The levels of FRAP were determined by using a spectrophotometer and comparing changes of optical absorption in 593-nm wave length. The results were expressed as micromole per gram kidney weight ($\mu\text{mol/g KW}$).

Statistical Analysis

The results were presented as mean \pm standard error and were analyzed using the SPSS software (Statistical Package for the Social Sciences, version 18.0, SPSS Inc, Chicago, Ill, USA). The 1-way analysis of variance and the Duncan post-hoc test were performed for intergroup comparisons of kidney functional parameters and oxidative stress values. A P value less than .05 was considered as the level of significance.

RESULTS

Tissue malondialdehyde and Ferric Reducing Antioxidant Power

No difference was found in the levels of malondialdehyde and FRAP between the control and sham groups. Gentamicin significantly increased the level of tissue malondialdehyde ($24.7 \pm 3.4 \text{ nmol/g KW}$) compared to that in the sham group ($13.6 \pm 2.6 \text{ nmol/g KW}$; $P < .001$; Figure 1). Treatment with calcium dobesilate (50 mg/kg and 100 mg/kg) could inhibit the increase of malondialdehyde induced by gentamicin ($P < .001$), such that the level of malondialdehyde in the group receiving calcium dobesilate reached its level in the sham group without any significant difference. However, the decrease in malondialdehyde in the D100 group was more than the decrease in the D50 group ($P < .05$). On the other hand, gentamicin administration led to a considerable reduction in the level of tissue FRAP in the gentamicin group ($4.6 \pm 0.3 \mu\text{mol/g KW}$) compared to the sham group ($8.5 \pm 1.1 \mu\text{mol/g KW}$; $P < .001$). Treatment with calcium dobesilate could increase the level of FRAP in both of the D50 and D100 groups (Figure 1).

Kidney Function Tests

No difference was found in the kidney function tests between the control and sham groups. Figure 2 showed that gentamicin led to a considerable increase in the concentration of plasma creatinine

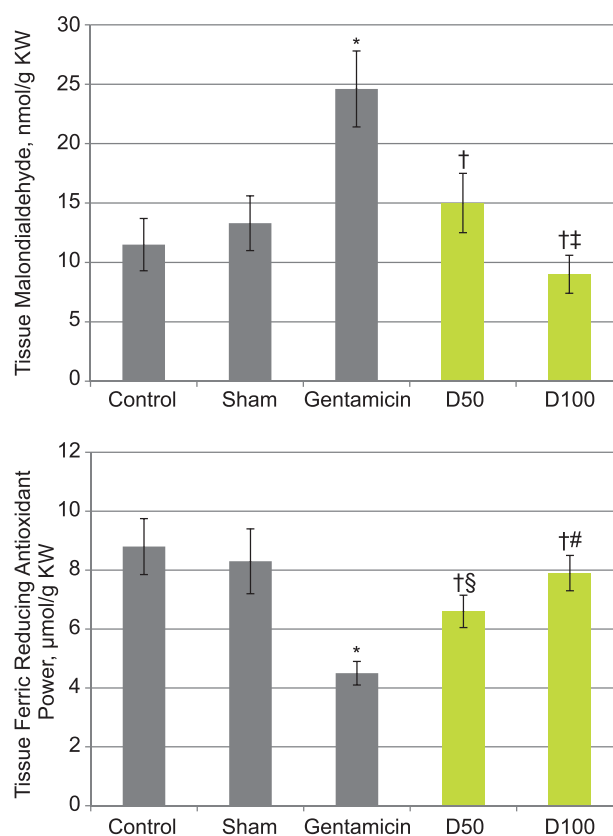


Figure 1. Tissue malondialdehyde and ferric reducing antioxidant power (FRAP) levels in rats received with gentamicin-induced nephrotoxicity pretreated with saline (gentamicin group), calcium dobesilate at a dose of 50 mg/kg/d (D50) and 100 mg/kg/d (D100) doses, and rats with sham surgery (sham) or without any operation (control).
 * $P < .001$ as compared to the sham group
 † $P < .001$ as compared to the gentamicin group
 ‡ $P < .05$ as compared to the calcium dobesilate 50 mg/kg/d group
 § $P < .01$ as compared to the sham group
 # $P < .05$ as compared to the calcium dobesilate 50 mg/kg/d group

($P < .001$) and urea nitrogen ($P < .01$) as compared to the sham group. Both doses of calcium dobesilate could significantly reduce the concentration of plasma creatinine compared to the gentamicin group ($P < .001$), such that it reached to its level in the sham group. In addition, administration of calcium dobesilate could significantly decrease the concentration of plasma urea nitrogen compared to the gentamicin group (Figure 2).

As seen in the Table, gentamicin considerably reduced the level of creatinine clearance compared to the sham group ($P < .001$). However, treatment with calcium dobesilate significantly increased the level of creatinine clearance compared to the gentamicin group ($P < .001$), so that it reached the level of creatinine clearance in the sham group. Absolute excretion of sodium in the gentamicin

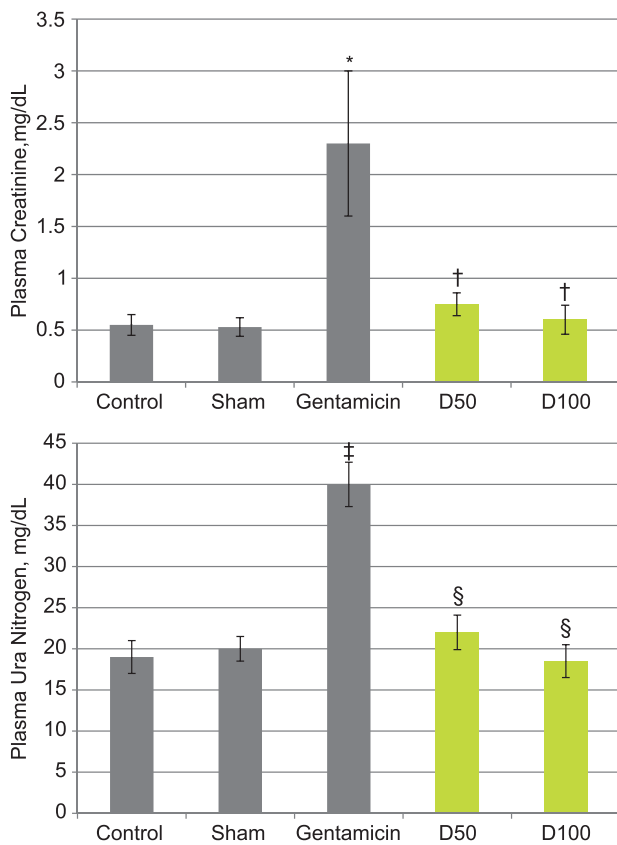


Figure 2. Plasma creatinine and urea nitrogen concentration in rats received with gentamicin-induced nephrotoxicity pretreated with saline (gentamicin group), calcium dobesilate at a dose of 50 mg/kg/d (D50) and 100 mg/kg/d (D100) doses, and rats with sham surgery (sham) or without any operation (control).
 * $P < .001$ as compared to the sham group
 † $P < .001$ as compared to the gentamicin group
 ‡ $P < .01$ as compared to the sham group
 § $P < .05$ as compared to the gentamicin group

group did not differ from the sham group and was not influenced by calcium dobesilate administration. Nevertheless, fractional excretion of sodium in the

gentamicin group grew significantly compared to the sham group ($P < .01$) and both doses of calcium dobesilate led to a decrease in the level of fractional excretion of sodium ($P < .001$) and reached it to a level equal to the sham group. Although there was a decline in absolute excretion of potassium in the gentamicin group, as compared to the sham group, this reduction was not significant. However, calcium dobesilate led to a considerable increase in absolute excretion of potassium in a dose-dependent fashion. In addition, fractional excretion of potassium in the gentamicin group increased compared to the sham group ($P < .05$) and insignificantly decreased by a 50 mg dose of calcium dobesilate. However, 100 mg of calcium dobesilate added to the fractional excretion of potassium again.

DISCUSSION

This study was conducted to investigate the effects of calcium dobesilate intake in prevention of gentamicin-induced nephrotoxicity in rats. As it was indicated, the level of oxidative stress, plasma parameters, and excretive function of kidneys did not differ in the control and sham groups. Therefore, it can be concluded that the stress caused by surgery could not bring about changes to the sham group compared to the control group.

Gentamicin is an aminoglycoside antibiotic that is widely used for the treatment of infections caused by gram-negative bacteria, but its use is restricted due to the possibility of acute kidney injury.²⁵ Researchers believe that the increase in the production of ROS by gentamicin is the key to the toxicity resulted from its administration.^{1,26} Gentamicin is actively reabsorbed in the proximal

Mean Changes in Kidney Function Parameters Following Gentamicin-induced Nephrotoxicity and Effect of Calcium Dobesilate Administration

Parameter	Control	Sham	Gentamicin Groups		
			No Calcium Dobesilate	Calcium Dobesilate, 50 mg/kg	Calcium Dobesilate, 100 mg/kg
Creatinine clearance, mL/min/kg	70.9 ± 6.35	65.70 ± 7.56	30.30 ± 4.05*	82.00 ± 4.89†	68.10 ± 3.72†
Absolute excretion of sodium, mmol/min/kg	1.73 ± 0.23	2.13 ± 0.73	1.93 ± 0.11	2.00 ± 0.11	1.80 ± 0.19
Fractional excretion of sodium, %	0.17 ± 0.02	0.18 ± 0.05	0.52 ± 0.04‡	0.19 ± 0.01†	0.17 ± 0.01†
Absolute excretion of potassium, mmol/min/kg	3.71 ± 0.68	3.91 ± 0.85	2.17 ± 0.31	5.10 ± 0.65†	7.00 ± 0.49†#
Fractional excretion of potassium, %	13.20 ± 2.31	11.30 ± 1.68	21.2 ± 4.03§	16.30 ± 2.64	24.00 ± 1.18‡

* $P < .001$ as compared to the sham group
 † $P < .001$ as compared to the gentamicin group
 ‡ $P < .01$ as compared to the sham group
 § $P < .05$ as compared to the sham group
 # $P < .05$ as compared to the calcium dobesilate 50 mg/kg/d group

convoluted tubule and its concentration in the tubular cells damages the proximal tubule and disturbs renal blood circulation, which in turn results in a reduction in GFR and an increase in the levels of plasma creatinine and urea nitrogen.²⁷ Comparison of the results of the gentamicin group with those of the sham group reveals a reduction in creatinine clearance (which reflects GFR level) in the gentamicin group. This leads to an increase in the concentration of plasma creatinine and urea nitrogen in this group (Table and Figure 1). Nevertheless, in spite of the decrease in GFR, the absolute excretion of sodium did not decrease considerably in the gentamicin group. The reason is reduced tubular reabsorption caused by tubular damages, and increased fractional excretion of sodium verifies this. Moreover, absolute excretion of potassium in the gentamicin group was decreased by 45% compared to the sham group due to the reduction in GFR. However, since potassium has tubular secretions, the reduction in its absolute excretion is less than the decrease in GFR. Tavafi and coworkers indicated that a 100 mg/kg/d of gentamicin in a 12-day period considerably increased plasma creatinine and urea nitrogen concentration, malondialdehyde level, and tubular necrosis, and decrease antioxidant enzymes.¹

Considerable increase in plasma creatinine and urea nitrogen is suggested as an index of gentamicin-induced nephrotoxicity,⁶ which was also documented in the present study. The levels of plasma creatinine and urea-nitrogen were reduced considerably in the group that simultaneously received calcium dobesilate and gentamicin compared to the gentamicin group. Several researchers have shown that involvement of antioxidant agents prevents the increase in the concentrations of creatinine and urea nitrogen following gentamicin administration.^{1,2} Furthermore, since calcium dobesilate is a strong antioxidant,¹⁶ it could probably prevent changes in the concentrations of creatinine and urea nitrogen due to its antioxidant effects. In 2001, a study that was conducted by Szabo and colleagues indicated that 100 mg/kg intraperitoneal dose of calcium dobesilate for 10 days could considerably protect the retina against oxidative stress caused by ischemia and reperfusion.²⁸ Furthermore, Ermelindo and colleagues found out in a study on rats that a 100 mg/kg/d dose of calcium dobesilate

for 10 days mitigated the retinopathy caused by diabetes. They concluded that the mechanism used by calcium dobesilate for preventing diabetic retinopathy might be the result of the inhibition of the activity of intracellular signaling pathways and transcription factors such as p38 MAPK and NF- κ B, by its antioxidant impacts.²⁹

It has been reported that gentamicin changes the lipid contents of renal cellular membranes and these changes are probably mediated by the lipid peroxidation activity of free radicals.¹ The level of malondialdehyde as an index of lipid peroxidation is increased in animals treated with gentamicin.³⁰ In the present study, the level of malondialdehyde in the gentamicin receiver group was increased considerably compared to the sham group. Different doses of calcium dobesilate (50 mg/kg/d and 100 mg/kg/d) reduced the malondialdehyde level. These results reflect the antioxidant properties of this drug.

Free radicals produced in the body are naturally removed by the antioxidant system of the body. When the production of ROS is increased or the antioxidant system of the body is weakened, ROS induce damages to tissues.³ Calcium dobesilate not only reduces free radicals, but also reinforces the antioxidant system of the body. This finding is consistent with the increase in the tissue FRAP in the group simultaneously treated by calcium dobesilate and gentamicin in comparison with group treated by gentamicin only. Creatinine clearance in the group treated with calcium dobesilate was higher than the group treated by gentamicin (Table). Increased creatinine clearance in this study shows the enhancement of glomerular function in the group treated with calcium dobesilate.

In addition to tubular necrosis, gentamicin has glomerular effects that disturb filtration. These effects include contraction of mesangial cells, loss of the selective barrier of glomerular filtration due to the neutralization of its negative charge, and necrosis of mesangial cells.³¹ Development of creatinine clearance by calcium dobesilate may be the result of the recovery of glomerular and tubular effects caused by gentamicin toxicity or the reduction in oxidative stress.

In 2009, in a study conducted by Park and associates on the effects of paricalcitol on the renal damage caused by gentamicin, it was indicated that administration of gentamicin for a 14-day period

leads to the reduction in urine osmolarity, increase of fractional excretion of sodium, increase in serum creatinine concentration, and decrease in creatinine clearance.³² In the present study, fractional excretion of sodium and potassium was increased and absolute excretion of potassium was decreased in the group treated with gentamicin only. These findings may reflect damages of the proximal tubule and thick ascending limb.¹² In the group that simultaneously received calcium dobesilate and gentamicin, the fractional excretion of sodium was similar to the sham group. This may be the result of the protective capability of calcium dobesilate for preventing gentamicin-induced oxidative stress. However, in spite of these effects of calcium dobesilate, the absolute excretion of potassium in the calcium dobesilate groups was increased dose dependently. Since the fractional excretion of potassium is also increased by 100 mg dose of calcium dobesilate, it may be caused by a reduction in reabsorption of potassium in the renal tubules.

CONCLUSIONS

In the present study, the protective effect of calcium dobesilate against gentamicin-induced nephrotoxicity was studied for the first time. The results of this study suggest that calcium dobesilate intake can mitigate the nephrotoxicity caused by gentamicin in rats. The reason can be that calcium dobesilate has antioxidant effects and reinforces the antioxidant system of the body. These findings prove the medical ability of calcium dobesilate, as a new nephroprotective agent, for defeating aminoglycosides. Nonetheless, further studies with larger sample sizes and longer follow-ups are required to verify these findings.

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CONFLICT OF INTEREST

None declared.

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