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RESEARCH ARTICLE

Protective Effect of Phytic Acid in Gentamycin Induced Nephrotoxicity in Rats

Shah PA[*1], *Gupta AD*[1], *Deshpande SS*[1]

[1]Department of Pharmacology and Pharmacy Practice, K. B. Institute of Pharmaceutical Education and Research, Gandhinagar, Gujarat, India.

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ABSTRACT

Gentamycin-induced nephrotoxicity mainly involves the generation of reactive oxygen species. Phytic acid is an abundantly found compound in various grains with antioxidant properties. We studied the protective effect of phytic acid against gentamicin-induced nephrotoxicity in rats. Male Sprague Dawley rats were divided into 4 groups each of 6 animals. All the animals except normal control received gentamycin (100 mg/kg, i.p, oid) from day 1 to 8. Group I and II served as normal and disease control respectively. Test groups III and IV received phytic acid 150 mg/kg and 300 mg/kg respectively (p.o, oid from day 1 to 8). Serum creatinine, serum uric acid (UA), blood urea nitrogen (BUN), urine volume (24 hr), urine creatinine, urine UA and total protein were measured. Creatinine clearance (CLCr), fractional excretion of UA (FEUA), and fractional excretion of sodium (FENa %) were calculated. Superoxide dismutase (SOD) and catalase (CAT) activity; malondialdehyde (MDA) and reduced glutathione (GSH) levels were measured in kidney homogenate. Phytic acid treatment significantly lowered the levels of serum creatinine, serum UA, BUN, urine creatinine, urine UA; and total protein in the urine as compared to disease animals. Phytic acid significantly increased GSH content, SOD and CAT activity; and lowered MDA level. Creatinine clearance and FEUA were significantly higher in treatment groups. Histopathological studies of the kidney showed less tubular, and glomerular damage and fewer signs of inflammation in treated groups. It can be concluded that phytic acid shows a protective effect in gentamycin-induced nephrotoxicity in rats due to its antioxidant property.

KEYWORDS

Nephrotoxicity, gentamycin, phytic acid, reactive oxygen species, antioxidant

INTRODUCTION

Nephrotoxicity is characterized by reduced glomerular filtration rate (GFR), elevated serum creatinine and blood urea nitrogen (BUN); and proteinuria. It is the major adverse effect of aminoglycoside antibiotics, especially gentamycin, account for 10 % to 15 % of all cases of acute renal failure. Gentamycin toxicity is due to its marked accumulation and retention in the proximal convoluted tubules1. Gentamycin mainly causes acute tubular necrosis. It is postulated that gentamycin may enhance the generation of reactive oxygen species (ROS) by damaging renal mitochondria. Gentamycin may alter mitochondrial respiration; generates superoxide anion and hydroxyl radical in renal mitochondria2, 3, 4. Gentamycin-iron complex

*Address for Correspondence:

Shah Palak A,

Dept. of Pharmacology and Pharmacy Practice,

K. B. Institute of Pharmaceutical Education and Research,

Sector-23, GH-6 Cross Roads, Gandhinagar-382023, Gujarat, India.

causes lipid peroxidation *In vitro* and is a potent catalyst for free-radical formation5. The role of ROS is well-established in gentamycin-induced nephrotoxicity. Studies showed that hydroxyl radical scavengers are protective in gentamicin-induced acute renal failure in rats 6,7. Co-administration of antioxidants, vitamin E, and selenium is protective against gentamycin-induced nephrotoxicity 8. Phytic acid (myo-inositol hexaphosphoric acid) is an abundant plant constituent, chiefly found in edible legumes, pollens, nuts, cereals, and seeds about almost 1-5% by weight. Phytic acid can chelate iron and it is a potent inhibitor of the iron-driven formation of reactive oxygen species 9. It has anticarcinogenic activity partly attributable to its antioxidant property 10. In this study, we evaluated the protective effect of phytic acid in gentamycin-induced nephrotoxicity in rats.

MATERIALS AND METHODS

Drugs and Chemicals

Gentamicin Sulphate Powder (Morvel Laboratory, Mehsana, Gujarat), 40 % phytic acid solution (purity 98 %) (Sigma Aldrich, St. Louis, USA); uric acid kit (Beacon Diagnostic Pvt. Ltd, Navsari, India), creatinine kit, BUN and total protein kit from (Span Diagnostic Pvt. Ltd, Surat, India) were purchased.

Ethics Approval

The project was approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the Institutional Animal Ethics Committee K. B. Institute of Pharmaceutical Education & Research, Gandhinagar; Project no. KBIPER/12/303.

Animals and study groups

Healthy male Sprague-Dawley rats (body weight 200 to 250 g) were selected and divided into 4 groups of 6 animals in each. The rats were acclimatized to standard condition of temperature (22 ± 1°C), relative humidity (40 to 60 %), and 12:12 hr alternate light & dark cycle. They were fed with the standard pellet diet (Pranav Agro Ltd, Gujarat, India) and water *ad libitum.* Nephrotoxicity was induced by administering gentamycin (100 mg/kg/day, i. p, once a day, days 1 to 8) in all animals except the normal control group. The normal control group (I) received a normal saline solution (0.5 mL, i. p). Group II served as the disease group and received gentamycin only. Test groups III and IV received phytic acid 150 mg/kg and 300 mg/kg respectively (p. o, once a day, from day 1 to 8).

Table 1: Groups and Treatments

Group	Groups	Treatment
I	Control	Normal Saline Solution (0.5 mL, i. p)
II	Disease	Gentamycin (100 mg/kg, i. p, once a day) from day 1 to 8
III	Phytic Acid 150 (Test I)	Gentamycin (100 mg/kg, i. p, once a day) + Phytic Acid (150 mg/kg, p. o, once a day) from day 1 to 8
IV	Phytic Acid 300 (Test II)	Gentamycin (100 mg/kg, i. p, once a day) + Phytic Acid (300 mg/kg, p. o, once a day) from day 1 to 8

Parameters:

Body weight, food, and water intake (24 hr) were measured (day1 & 8). The blood was collected from retro orbital plexus of each animal using isoflurane inhalation anaesthesia (day 1 & 8). The sample was centrifuged at 4000-5000 rpm for 15 minutes and the serum was separated. Serum uric acid (UA), creatinine & blood urea nitrogen (BUN) were estimated. Urine was collected from each animal on day 1 & 8 (at 24 hr). Urine volume & pH of urine (using pH strip)

were measured. Uric acid, creatinine & total protein were estimated in urine. Creatinine clearance (CLCr), fractional excretion of UA (FEUA), and fractional excretion of sodium (FENa %) were calculated. On day 8, all the animals were sacrificed using a carbon dioxide chamber. The kidney was isolated, washed with saline, and weighed. It was further processed for histopathology. Levels of reduced glutathione (GSH)[11] and malondialdehyde (MDA)[12], superoxide dismutase (SOD)[13,] and catalase (CAT) activity[14] were measured in kidney homogenate.

Statistical Analysis

Data were expressed as mean ± SEM (n=6). P<0.05 was considered statistically significant. One-way Analysis of Variance (ANOVA) was carried out followed by the post hoc Tukey test. Statistical analysis was performed using Sigma Plot for Windows version 12 software developed in 2011.

RESULTS AND DISCUSSION

Percentage change in body weight (%)

Animals in disease group (-44.02±4.32) showed significant % change (reduction) in body weight (BW) as compared to control group (17.29±1.42) on day 8. Percentage change in BW was significantly different in animals treated with phytic acid 150 mg and 300 mg (6.48±1.42 and 15.57±3.17 respectively) as compared to the gentamycin group (Fig. 1).

Food intake (gm)

Animals in the disease group (16.16±0.30) showed significantly lower food intake as compared to the control group (22.83±0.60) on day 8. Animals treated with phytic acid 150 mg and 300 mg showed significantly higher food intake (23.16±0.60 and 24±0.60 respectively) as compared to the disease group on day 8 (Fig. 2).

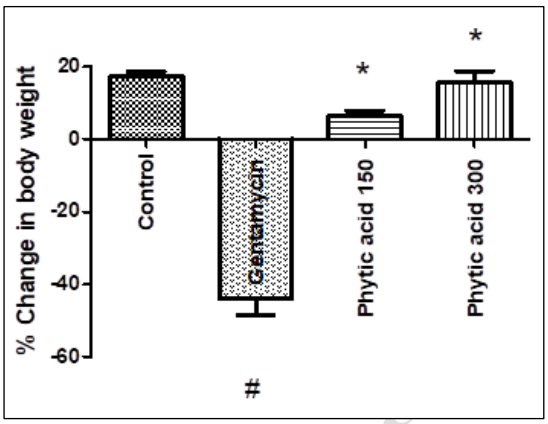

Figure 1: Effect of Phytic Acid on % Change in Body Weight

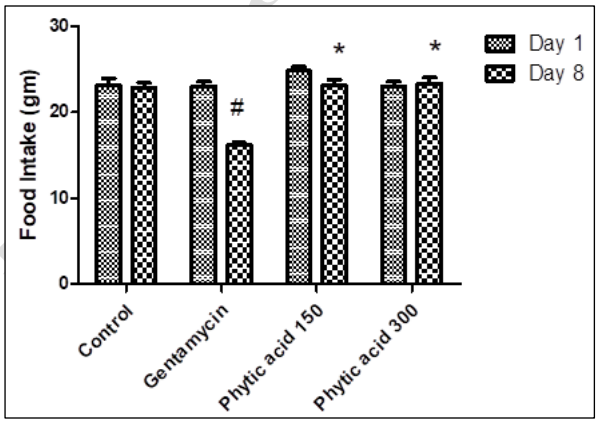

Figure 2: Effect of Phytic Acid on Food Intake (gm). # indicates the significant difference from normal control, * indicates the significant difference from gentamycin (disease) group, (p<0.05, One way ANOVA followed by Tukey test)

Water intake, urine volume, and urine pH

No significant differences were found amongst normal, disease, and treatment groups.

Kidney weight (gm)

In the gentamycin group, kidney weight (0.56±0.03) was significantly lower as compared to the control group (0.78±0.02). Concurrent administration of phytic acid (150 and 300 mg/kg) with gentamycin showed significantly higher kidney weight (0.75±0.05 and 0.83±0.02 respectively) as compared to the gentamycin group (Fig. 3).

Blood urea nitrogen (BUN) (mg/dL)

BUN level was significantly higher in the gentamycin group (8.35±0.26) as compared to the control group (0.69±0.06) on day 8. Concurrent administration of phytic acid (150 and 300 mg/kg) with gentamycin significantly decreased BUN level (5.40±0.15 and 5.37±0.17 respectively) as compared to the gentamycin group respectively (Fig. 4).

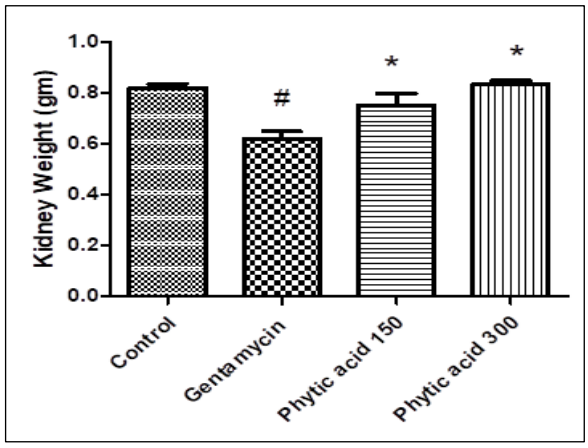

Figure 3: Effect of Phytic Acid on Kidney Weight (gm)

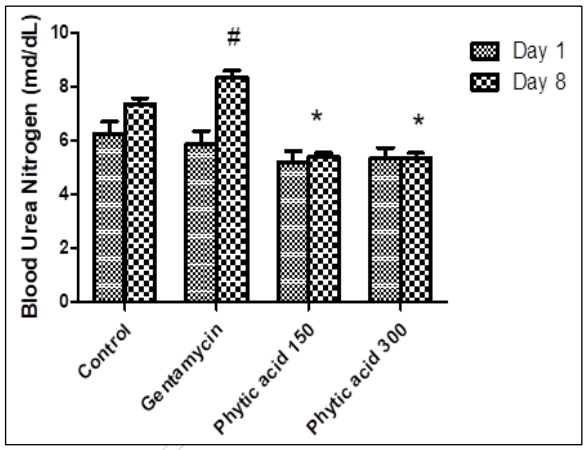

Figure 4: Effect of Phytic Acid on Blood Urea Nitrogen (mg/dL)

[#] indicates the significant difference from normal control, * indicates the significant difference from the gentamycin (disease) group, ($p<0.05$, One way ANOVA followed by Tukey test)

Serum and urine creatinine (mg/dL)

Serum and urine creatinine were significantly higher in the gentamycin group as compared to the control group. Concurrent administration of phytic acid (150 and 300 mg/kg) with gentamycin showed significantly decreased levels of serum and urine creatinine as compared to the gentamycin group. Phytic acid 300 mg/kg treatment significantly lowered serum creatinine levels as compared to 150 mg/kg dose (Table 2).

Table 2: Effect of Phytic Acid on Serum and Urine Creatinine (mg/dL)

Parameter	Day	Normal Control	Gentamycin	Phytic acid (150 mg/kg)	Phytic acid (300 mg/kg)
Serum creatinine	Day 1	0.60±0.06	0.48±0.06	0.57±0.07	0.52±0.07
	Day 8	0.69±0.06	0.69±0.06[#]	0.74±0.04[*]	0.67±0.04[*$]
Urine creatinine	Day 1	0.48±0.05	0.58±0.06	0.67±0.03	0.54±0.06
	Day 8	0.45±0.08	1.15±0.08[#]	0.63±0.06[*]	0.59±0.06[*]

[#] indicates the significant difference from normal control, * indicates the significant difference from *the* gentamycin (disease) group, [$] indicates the significant difference from test group I. ($p<0.05$, One way ANOVA followed by Tukey test).

Serum and urine uric acid (mg/dL)

Serum and urine uric acid were significantly higher in the gentamycin group as compared to the control group on day 8. Concurrent administration of phytic acid (150 and 300 mg/kg) with gentamycin significantly decreased serum and urine uric acid levels as compared to the gentamycin group (Table 3).

Table 3: Effect of Phytic Acid on Serum and Urine Uric Acid (mg/dL)

Parameter	Day	Normal Control	Gentamycin	Phytic acid (150 mg/kg)	Phytic acid (300 mg/kg)
Serum uric acid	Day 1	3.13±0.12	3.21±0.34	3.25±0.25	3.13±0.26
	Day 8	3.55±0.12	4.67±0.26#	3.51±0.27*	2.70±0.26*
Urine uric acid	Day 1	4.29±0.24	3.65±0.13	4.26±0.21	3.89±0.26
	Day 8	4.07±0.27	5.85±0.17#	4.10±0.18*	3.74±0.17*

# indicates the significant difference from normal control, * indicates the significant difference from *the* gentamycin (disease) group, ($p<0.05$, One way ANOVA followed by Tukey test).

Urine total protein (g/dL)(24hr)

Urine total protein was significantly higher in the gentamycin group (38.26±2.62) as compared to the control group (19.12±2.25) on day 8. Concurrent administration of phytic acid (150 and 300 mg/kg) with gentamycin significantly decreased total protein level in urine (22.07±1.19 and 19.52±1.15 respectively) as compared to the gentamycin group (Fig. 5).

Creatinine clearance (mL/min)

Creatinine clearance (CLcr) was significantly lower in the gentamycin group (0.20±0.03) as compared to the control group (0.59±0.06) on day 8. Concurrent administration of phytic acid (150 and 300 mg/kg) with gentamycin significantly increased CLcr (0.70±0.02 and 0.71±0.02 respectively) as compared to the gentamycin group (Fig. 6).

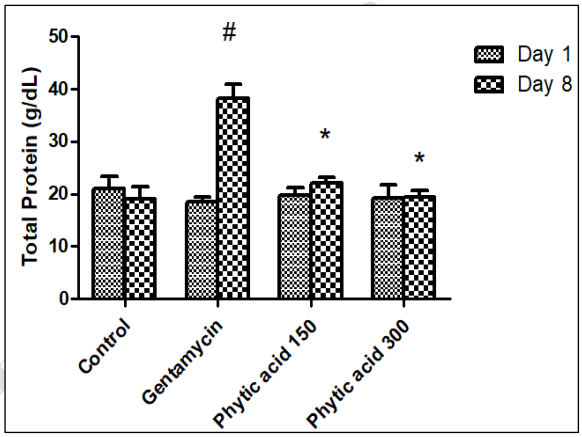

Figure 5: Effect of Phytic Acid on Urine Total Protein (g/dL)

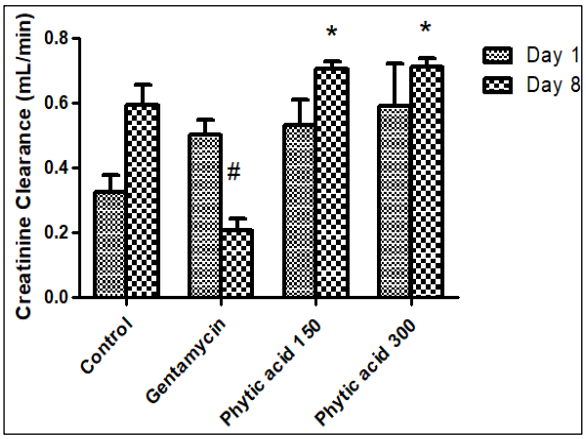

Figure 6: Effect of Phytic Acid On Creatinine Clearance (mL/min)

# indicates the significant difference from normal control, * indicates the significant difference from the gentamycin (disease) group. ($p<0.05$, One way ANOVA followed by Tukey test).

Fractional excretion of uric acid (FEUA) and sodium (FENa) (%)

FEUA was significantly lower in the gentamycin group as compared to the control group on day 8. Concurrent administration of phytic acid (150 and 300 mg/kg) with gentamycin significantly increased FEUA as compared to the gentamycin group respectively (Table 4). Fractional excretion of sodium (FENa) was significantly higher in the gentamycin group as compared to the control group on day 8. Concurrent administration of phytic acid (150 & 300 mg/kg) with gentamycin significantly decreased FENa as compared to the gentamycin group (Table 4).

Table 4: Effect of Phytic Acid on Fractional Excretion of Uric Acid (FEUA) and Sodium (FENa) (%)

Parameter	Day	Normal Control	Gentamycin	Phytic acid (150 mg/kg)	Phytic acid (300 mg/kg)
Fractional ex. of uric acid	Day 1	176.04± 19.80	100.21± 16.38	115.08± 17.56	144.66± 40.87
	Day 8	274.07± 52.83	126.5± 21.04#	246.56± 14.58*	255.86± 38.08*
Fractional ex. of sodium	Day 1	521.04± 16.32	491.96± 30.06	490.18± 35.21	484.73± 39.61
	Day 8	370.92± 41.56	530.48± 16.02#	351.86± 87.55*	326.07± 44.11*

# indicates the significant difference from normal control, * indicates the significant difference from gentamycin (disease) group, ($p<0.05$, One way ANOVA followed by Tukey test).

Effect of phytic acid on total protein in kidney, GSH level, MDA level, SOD and CAT activity (day 8) (Table 5)

The protein level in the gentamycin group was significantly lower than in the control group. Administration of phytic acid (150 and 300 mg/kg) significantly increased protein levels in the kidney as compared to the gentamycin group. Total protein in the kidney was significantly higher in phytic acid 300 mg dose as compared to 150 mg dose. The level of GSH was significantly decreased and MDA was increased the in gentamycin group. Treatment with phytic acid 150 and 300 mg increased the levels of GSH and decreased MDA. Similarly, SOD and Catalase activity were significantly decreased in the gentamycin group. The activity of both enzymes was increased in phytic acid-treated groups.

Table 5: Effect of Phytic Acid on Total Protein in Kidney, GSH level, MDA level, SOD and CAT Activity (day 8)

Test	Normal Control	Gentamycin	Phytic acid (150 mg/kg)	Phytic acid (300 mg/kg)

Total protein in kidney (g/dL)	17.69±1.20	7.12±1.33[#]	41.77±4.39[*]	60.27±3.82[*$]
Reduced glutathione (GSH) (µg/mg protein)	355.44±30.75	188.47±15.81[#]	346.10±47.73[*]	381.62±29.25[*]
Malondialdehyde (MDA) (nmoL/mg protein)	572.06±137.98	1446.83±210.44[#]	676.75±63.21[*]	511.87±31.64[*]
Superoxide dismutase (SOD) (units/mg protein)	0.13±0.02	0.05±0.01[#]	0.15±0.02[*]	0.15±0.02[*]
Catalase (CAT) (units/mg protein)	0.20±0.02	0.07±0.01[#]	0.25±0.04[*]	0.20±0.02[*]

[#] indicates the significant difference from normal control, * indicates the significant difference from the gentamycin (disease) group, [$] indicates the significant difference from test group I. ($p<0.05$, One way ANOVA followed by Tukey test).

Histopathology of kidney (Magnification 100x × 40X)

Normal control (a) shows normal architecture in the tubular and glomerular parts in the cortex and medulla and shows normal papilla. There is no sign of inflammation or tissue damage. Gentamycin group (b) shows the presence of only renal glomeruli with loss of renal epithelial cells and increased vascularity. Phytic acid 150 (c) and phytic acid 300 (d) show almost intact glomeruli as well as normal tubular epithelial cells (Fig. 7).

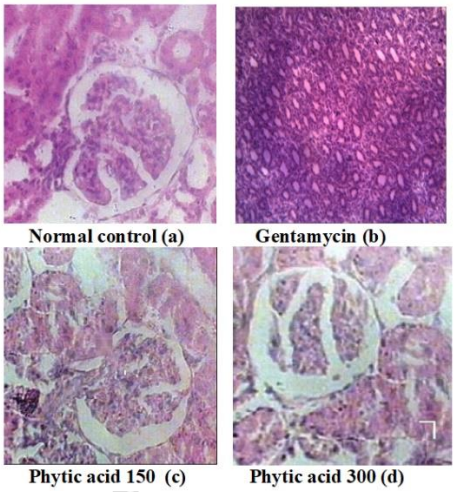

Figure 7: Histopathology of kidney

DISCUSSION

The results showed that administration of Gentamycin induced nephrotoxicity in rats. This was demonstrated by decreased body weight, kidney weight, food intake and CLcr; increased serum creatinine, BUN and uric acid levels; decreased protein content in kidney and increased urine protein levels. Oxidative stress was increased shown by decreased GSH level, increased SOD and Catalase activity; and increased lipid peroxidation. Phytic acid improved the markers of nephrotoxicity and improved antioxidant parameters. It has been proven that phytic acid promotes the oxidation of Fe2+ to Fe3+ which results in less Fe2+ available for hydroxyl radical formation 15. It is found to be affecting pathological targets in Alzheimer's disease [16]. Phytate can chelate iron, zinc, copper, and magnesium which are involved in ROS [17]. In our study, results show that phytic acid treatment improved antioxidants in the kidney and showed a nephroprotective effect.

CONCLUSION

Phytic acid decreased creatinine, BUN, uric acid, and urine protein excretion. It improved total protein in the kidney, creatinine clearance, FEUA, and FENa. It improved antioxidant parameters along with histopathological changes

induced due to gentamycin. It can be concluded that phytic acid has a nephroprotective effect due to its antioxidant activity.

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