

Evaluation of Chemoprotective Role of N-Acetylcysteine and Vitamin E on Gentamicin-Induced Nephrotoxicity

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Abstract: Gentamicin (GM) is widely used for treatment of severe gram negative infections; however its clinical use is partially limited due to its nephrotoxicity. This study aimed to evaluate the protective role of N-acetylcysteine (NAC) and vitamin E on gentamicin-induced nephrotoxicity. Forty adult male albino rats were divided into equal five groups, Group1: normal control administered standard diet for four weeks. Group2: administered standard diet for four weeks and injected with gentamicin (80 mg/kg body wt I.P) for the last 6 days. Group 3, 4 and 5: were administered standard diet, treated with (40 mg/kg body wt/day P.O.) of either N-acetylcystein or vitamin E or combination of them, respectively for four weeks and injected with gentamicin for the last 6 days. Gentamicin caused elevation of blood urea nitrogen, serum creatinine, urinary protein, gamma glutamyl transferase (GGT) and lactate dehydrogenase activities. Also renal malondialdehyde and nitric oxide were significantly increased. Serum (total protein and albumin) as well as renal reduced glutathione, GGT and alkaline phosphatase activities were decreased. Histopathological examination of kidneys demonstrated prominent damage in gentamicin treated rats. Treatment with NAC and/or vitamin E reduced lipid peroxidation, protected kidney from tubular damage and decrease nephrotoxicity. Vitamin E in combination with NAC was the most effective.

Key words: Gentamicin, N-acetylcysteine, vitamin E, nephrotoxicity.

INTRODUCTION

Gentamicin (GM), a widely used aminoglycoside antibiotic, recognized to possess significant nephrotoxic potential in man and experimental animals (Laurent *et al.*, 1990). It has been suggested that GM nephrotoxicity is primarily related to preferential accumulation in renal proximal convoluted tubules (Abdel-Naim *et al.*, 1999).

Experimental gentamicin nephrotoxicity has been investigated in various animal models so far, and several approaches considering different mechanisms have been attempted to reduce the nephrotoxicity of gentamicin and related aminoglycosides (Ali, 2003). Among these, the most consistent effect has been observed with the use of antioxidant agents such as vitamin C and vitamin E (Derakhshanfar *et al.*, 2007).

Vitamin E is a putative radical scavenger which is probably the most important inhibitor of membrane lipid peroxidation. It is a lipid soluble agent which can readily cross cell membranes and exert its effects both intracellularly and in membranes (Halliwell and Gutteridge, 1999).

Vitamin E can be safely used in high doses in the prevention of diabetes and cardiovascular disease (Czernichow and Hercberg, 2001). It suppresses the oxidative stress of membranes. Given to potential role of the reactive oxygen species (ROS) in mediating tissue damage, cells contain the enzymes, superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), which are important components of several naturally occurring antioxidant defense mechanisms to prevent oxidative injury (Weijl *et al.*, 1997).

N-acetylcysteine (NAC) is a precursor of the amino acid L-cysteine and helps glutathione synthesize pathway (Tepel *et al.*, 2000). The benefit of N-acetylcysteine for the prevention of contrast-induced nephropathy was first reported by Tepel *et al* in 2000. In a randomized controlled trial, the investigators found that N-acetylcysteine was associated with a decreased incidence of contrast-induced nephropathy compared with placebo in patients with renal insufficiency undergoing contrast-enhanced computed tomography (Trivedi *et al.*, 2009).

NAC acts as an antioxidant by restoring the pool of intracellular reduced glutathione, which is often depleted as a consequence of increased status of oxidative stress and inflammation. Furthermore, NAC also has reducing and antioxidant properties, acting as a direct scavenger of ROS (Goncalves *et al.*, 2010).

The present study was undertaken to examine the effects of gentamicin on various enzyme systems in the blood and kidney, and relate these effects to renal failure. It is also intended to evaluate the possible ways of reversing these effects by administration of compounds that protect or increase SH groups such as N-acetylcysteine and vitamin E.

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MATERIALS AND METHODS

Materials:

Chemicals:

N-acetylcysteine and vitamin E were obtained from British drug Houses (BDH) and Sigma-Aldrich. Co., respectively. Gentamicin ampoules as sulphate were provided by Memphis Co. for Pharm. and Chem. Ind. Cairo, Egypt.

Experimental Animals:

Forty male Sprague Dawley albino rats weighing (82-117 g) were purchased from Egyptian Organization for Biological Products and Vaccines (Hellwean farm). They were fed on standard laboratory diet prepared following defined composition of the AIN-93M (Reeves *et al.*, 1993) for one week to acclimatize. Food and water were offered *ad-libitum*. Rats were housed in individual cages at room temperature ($30 \pm 2^{\circ}\text{C}$) on 12-hr light dark cycle.

Methods:

Experimental Design:

Animals were classified into five groups, each one (8 rats) as follows:

Group 1: (Control) fed on standard diet for four weeks.

Group 2: (GM) fed on standard diet for four weeks and injected interperitonally (i.p.) with gentamicin (80mg/kg b. wt/day) for the last 6 days (Ali *et al.*, 2009).

Group 3: (NAC + GM) fed on standard diet and received oral dose of N-acetylcysteine (40 mg/kg b.wt/day) (Tariq *et al.*, 1999) for four weeks and injected i.p. with gentamicin for the last 6 days.

Group 4: (Vit. E + GM) fed on standard diet and received oral dose of vitamin E (40 mg/kg b.wt/day) (Moawad, 2007) for four weeks and injected with gentamicin for the last 6 days.

Group5: (NAC + Vit. E + GM) fed on standard diet and received oral dose of N-acetylcysteine and vitamin E for four weeks and injected with gentamicin for the last 6 days.

At the end of the experimental period (4 weeks) and after the last injection, animals were kept in wire bottom stainless steel metabolic cages for the collection of 24-hour urine samples. Urine specimens were filtered, the filtrate were divided into aliquots and stored at -20°C until urine analysis. Animals were sacrificed after overnight fasting and blood samples were taken from the portal vein, blood was allowed to coagulate and centrifuged at 3000 rpm for 20 min. to separate serum which was kept frozen at -20°C until used for biochemical analysis. Kidneys were removed, washed with physiological saline (0.9% W/V), dried between filter paper.

Part of kidney was used for the determination of reduced glutathione, malondialdehyde as well as nitric oxide, alkaline phosphatase and gamma glutamyl transferase activities. Other part was dipped in 10% buffered formalin for histopathological assessment.

Biochemical Analysis:

Blood urea nitrogen (BUN) and urine urea were analyzed using kits from Bioanalytics Company following the method described by Tabacco *et al.* (1979). Serum and urine creatinine concentrations were analyzed using kits from Bioanalytics Company following the method described by Fabing and Ertingshausen (1971). Serum total protein and albumin were determined according to the methods described by Weichselbaum (1946) and Dumas *et al.* (1971), respectively.

Alkaline phosphatase (Alk-P) was determined in serum and kidney by enzymatic colorimetric method described by Haussament (1977). Gamma glutamyl transferase (GGT) activity was determined in serum, kidney and urine according to the method described by Rosalki (1975). Lactate dehydrogenase (LDH) activity was determined in serum and urine by the method described by Mc Queen (1972). Reduced glutathione (GSH), malondialdehyde (MDA) and nitric oxide (NO) were determined in kidney by the methods described by Beutler *et al.*, (1963), Draper and Hadley (1990) and Miranda *et al.* (2001), respectively.

Protein in urine was determined by the method described by Tietz (1994). Uric acid was determined in serum and urine by the method described by Fossati *et al.* (1980).

Histopathological Examination:

Kidney tissues were fixed in 10% buffered formalin and processed for preparation of 5 μ thickness and were stained with Haematoxylin and Eosin (H&E) according to Bancroft *et al.* (1996).

Statistical Analysis:

The data were statistically analyzed using one way analysis of variance (ANOVA) according to Bailey (1995). Values of ($P < 0.05$) were considered statistically significant.

Results:

Administration of gentamicin to rats resulted in significant increase ($P < 0.05$) in blood urea and serum (creatinine and uric acid) levels as compared to the corresponding control values indicating renal function insufficiency (Table 1).

N-acetylcysteine or vitamin E given each alone or in combination for four weeks to the gentamicin injected rats reduced the elevation of these parameters.

Gentamicin administration caused no significant change in serum ALK-P, LDH and GGT activities while induced a significant reduction in serum total protein and albumin ($P < 0.05$). Pre-treatment with vitamin E in combination with N-acetylcysteine caused a significant elevation of serum total protein ($P < 0.05$).

Table 1: Effect of N-acetylcysteine and/or vitamin E on some biochemical parameters in serum of adult male albino rats treated with gentamicin

Parameters	BUN (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)	T. protein (g/dl)	Albumin (g/dl)	Alk-P activity (U/L)	LDH activity (U/L)	GGT activity (U/L)
Control	35.81±0.53 ^a	0.79±0.52 ^a	1.97±0.12 ^a	7.10±0.42 ^a	2.92±0.23 ^a	213.12±11.22 ^a	406.91±8.79 ^a	12.73±0.65 ^a
GM	72.61±1.03 ^b	2.97±0.38 ^b	2.95±0.21 ^b	5.99±0.80 ^b	2.13±0.19 ^b	208.28±11.66 ^a	396.66±8.95 ^a	12.79±2.83 ^a
NAC+GM	70.47±1.58 ^b	1.99±0.22 ^c	1.81±0.13 ^a	6.39±0.75 ^{ab}	2.41±0.15 ^{ab}	207.74±10.02 ^a	385.28±19.65 ^a	14.21±0.44 ^a
Vit. +GM	69.60±1.44 ^b	2.09±0.26 ^c	1.74±0.14 ^a	6.64±0.29 ^{ab}	2.55±0.19 ^{ab}	232.54±15.97 ^a	389.34±7.89 ^a	14.12±0.45 ^a
NAC + Vit.E + GM	59.44±1.28 ^c	1.84±0.33 ^c	1.71±0.10 ^a	7.00±0.23 ^a	2.49±0.25 ^{ab}	209.66±2.57 ^a	383.05±17.69 ^a	13.75±0.29 ^a

Values represent (Mean ± SE), n = 8 rats.

Values followed by the different superscript letter within the same column are significantly different at ($P < 0.05$).

Results tabulated in table (2) showed that gentamicin induced a considerable increase in kidney nitric oxide and malondialdehyde as compared to controls. Vitamin E alone or with N-acetylcysteine caused a significant decrease of these parameters. Administration of N-acetylcysteine alone significantly reduced kidney MDA only. On the other hand significant decreases were also recorded in activities of kidney alkaline phosphatase and gamma glutamyl transferase. Reduced glutathione decrement was noted after injection of gentamicin (80 mg/kg b.wt for 6 days), and an amelioration happened in groups administered N-acetylcysteine or vitamin E or both of them prior to gentamicine injection.

Table 2: Effect of N-acetylcysteine and/or vitamin E on some biochemical parameters in kidney of adult male albino rats treated with gentamicin

Parameters	Nitric oxide activity (μmol/g)	Alk-P activity (u/g)	GSH (mg/g)	MDA (mmol/g)	GGT activity (u/g)
Control	4.08±0.09 ^a	718.36±3.38 ^a	172.50±1.22 ^a	1.12±0.03 ^a	156.96±1.74 ^a
GM	6.23±0.19 ^b	484.59±12.00 ^b	140.79±2.08 ^b	1.46±0.03 ^b	105.08±2.22 ^b
NAC +GM	5.99±0.12 ^b	589.23±12.29 ^c	167.43±2.24 ^c	1.14±0.03 ^a	130.7±2.25 ^c
Vit. E+GM	4.34±0.16 ^a	709.49±6.26 ^c	170.19±2.19 ^{ac}	1.01±0.01 ^c	151.35±2.22 ^a
NAC + Vit.E + GM	4.20±0.22 ^a	619.31±8.76 ^d	169.63±1.49 ^{ac}	1.02±0.01 ^c	154.90±1.40 ^a

Values represent (Mean ± SE), n = 8 rats.

Values followed by the different superscript letter within the same column are significantly different at ($P < 0.05$).

NAC and vitamin E significantly ameliorated some effects on the kidney caused by gentamicin injection. Each of them alone or in combination protected rats from elevated creatinine, uric acid and protein in urine (Table 3). NAC and/or vitamin E given prior gentamicin led to significant decrease in urine GGT and LDH activities.

Table 3: Effect of N-acetylcysteine and/or vitamin E on some biochemical parameters in urine of adult male albino rats treated with gentamicin

Parameters	Urea (g/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)	Protein (mg/dl)	GGT (U/L)	LDH (U/L)
Control	31.86±1.35 ^a	12.44±0.40 ^a	216.71±1.15 ^a	15.82±0.10 ^a	11.42±0.26 ^a	213.74±0.40 ^a
GM	41.00±1.50 ^b	14.54±0.32 ^b	274.47±1.14 ^b	24.91±0.09 ^b	13.57±0.22 ^b	293.92±0.98 ^b
NAC +GM	40.71±1.46 ^b	12.93±0.08 ^a	261.79±0.88 ^c	21.45±0.29 ^c	10.48±0.18 ^c	233.82±0.68 ^c
Vit. E+GM	40.00±1.69 ^b	12.77±0.07 ^a	241.54±1.14 ^d	21.75±0.25 ^c	10.53±0.19 ^c	225.85±0.61 ^d
NAC +Vit.E + GM	39.13±0.94 ^b	11.19±0.17 ^c	228.57±1.25 ^c	23.71±0.23 ^d	10.86±0.12 ^{ac}	216.17±1.53 ^a

Values represent (Mean ± SE), n = 8 rats.

Values followed by the different superscript letter within the same column are significantly different at ($P < 0.05$).

Microscopic histopathological examination of renal sections of rats revealed normal structural pattern of renal parenchyma in normal rats (group 1), (fig.1-a). Rats treated with gentamicin (group 2) showed tubulointerstitial nephritis as well as interluminal eosinophilic proteinaceous cast (fig.1-b).

Kidney of rats that received N-acetylcysteine or vitamin E before gentamicin (group 3 and 4) showed necrosis of renal tubules with interstitial leucocytic cells infiltration (fig. 1-c) and intratubular eosinophilic protein cast (fig. 1-d), respectively.

Microscopic observations of kidney of rats (group 5) which received combination of N-acetylcysteine and vitamin E before gentamicin injection revealed normal renal parenchyma (fig. 1-e).

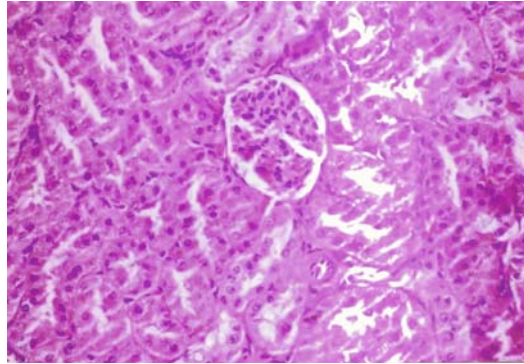


Fig. 1-a: Kidney of group 1 showing normal structure of renal parenchyma (H & E \times 400).

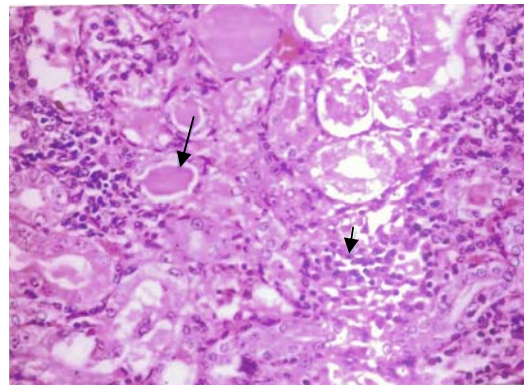


Fig. 1-b: Kidney of group 2 have revealed tubulointerstitial nephritis as well as intraluminal eosinophilic proteinaceous cast (H & E \times 400).

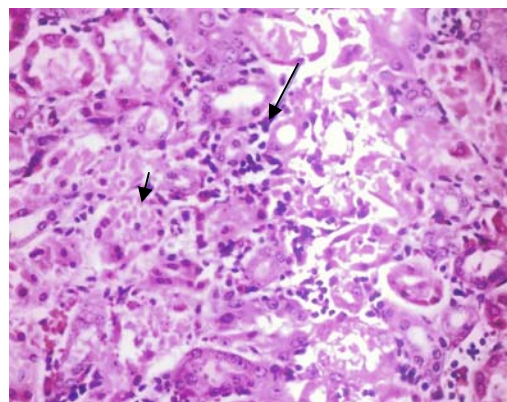


Fig. 1-c: Kidney of group 3 showing mild necrosis of renal tubules with interstitial leucocytic cells infiltration (H & E \times 400).

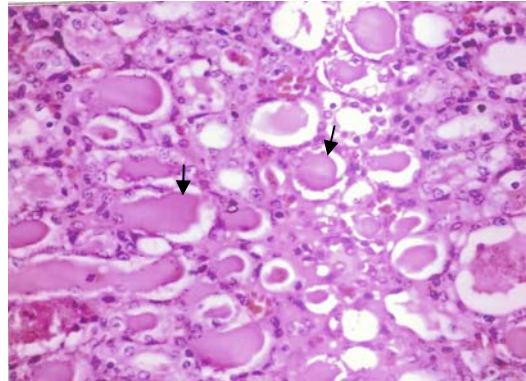


Fig. 1-d: Kidney of group 4 intratubular eosinophilic protein cast can be seen (H & E \times 400).

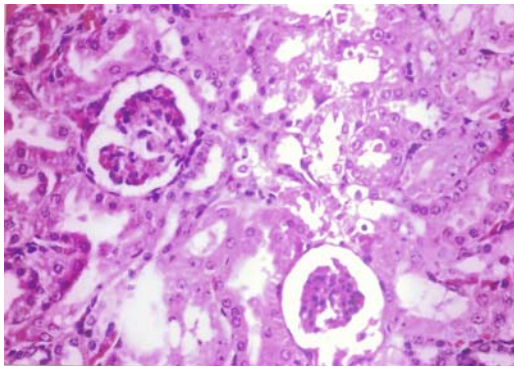


Fig. 1-e: Kidney of group 5 showing apparent normal renal parenchyma (H & E \times 400).

Discussion:

Nephrotoxicity of aminoglycoside antibiotics, and specially that of the most commonly used compound, gentamicin is well documented (Laurent *et al.*, 1990). As expected all rats injected with gentamicin (80 mg/ kg b.wt) for sex days showed a decrease in glomerular filtration indicated by elevated levels of serum creatinine, uric acid and BUN, and increased urinary enzymatic activity of GGT as previously shown by Laurent *et al.* (1990) and Derakhshanfar *et al.* (2007).

In the current study administration of N-acetylcysteine and/or vitamin E pre-treatment with gentamicin caused a marked protection against nephrotoxicity indicated by a significant relative reduction in BUN and serum creatinine compared to the untreated group. Abdin *et al.* (2008) found that administration of N-acetylcysteine one hour after cisplatin offered marked protection against nephrotoxicity. This protection was manifested as significant relative reduction in serum levels of urea and creatinine, amelioration of both apoptotic markers caspase-3 and DNA fragmentation as well as the histopathological changes.

NAC was able to reduce the severity of renal dysfunction induced by ifosfamide with a significant decrease in elevations of serum creatinine (Chen *et al.*, 2008).

Ten days of treatment with gentamicin produced remarkable nephrotoxicity, characterized by an increase in BUN and significant decrease in the creatinine clearance as compared with the control. Pre-treatment of rats with vitamin E resulted in marked changes in nephrotoxicity in day 10 represented by significant changes in BUN concentration (Sener *et al.*, 2003 and Derakhshanfar *et al.*, 2007).

The protective effect of vitamin E against the nephrotoxic symptoms of gentamicin was previously reported. Vitamin E significantly inhibited the rise in serum urea and creatinine as well as the increase in urinary activity of GGT in rats which administered single dose of gentamicin (150mg/kg b.wt i.p) (Abd-EL- Naim *et al.*, 1999).

In our study, gentamicin induced significant decrease in both serum total protein and albumin with a significant increase in urinary protein. These results were in harmony with the results of El-Daly (1996) who reported that significant proteinuria was seen in the cisplatin-treated rats. This is further supported by the lower serum albumin levels in the treated rats.

Renal oxidative stress was shown in rats represented by decreased kidney content of GSH and increased kidney level of MDA in the group treated with gentamicin. This is in harmony with the finding reported by Abd EL-Naim *et al.* (1999) and Derakhshanfar *et al.* (2009).

Administration of N-acetylcysteine and vitamin E prior to gentamicin inhibited the gentamicin-induced decrease in the enzyme activities. Vitamin E is the main fat-soluble endogenous antioxidant which reacts with oxygen radicals preventing free radical chain reactions thus protects the cell membranes.

Ramsammy *et al.* (1987) showed that the pre-treatment with antioxidants such as vitamin E drastically facilitated diffusion of this vitamin into lysosomal area, reduced the lipid peroxidation and MDA thus inducing shift from polyunsaturated to saturated fatty acids in the biological membranes.

Kadkhodae *et al.* (2007) mentioned that antioxidant vitamins have a role in preservation of renal endogenous antioxidant activities in gentamicin-induced nephrotoxicity.

It was found that moderate selenium and high dose of vitamin E supplementation may play a role in cisplatin-induced nephropathy (Naziroglu *et al.*, 2004). NAC is a thiol agent, acting as precursor of L-cysteine and glutathione pathway, although it is currently viewed as an antioxidant scavenging free radicals (Zafarullah *et al.*, 2003 and Abdin *et al.*, 2008).

Kidney alkaline phosphatase and gamma glutamyl transferase enzymes activities were reduced in gentamicin treated rats, N-acetylcysteine, vitamin E or combination of them corrected this state. Renal failure could be a result of the diminished enzymatic activities in the kidney, as well as the less efficient oxidative phosphorylation and adenosine triphosphate production of the mitochondria, this has been reported in cisplatin-treated rats. The enzyme shown to be most inhibited both in serum and kidney is alkaline phosphatase which involved in the renal failure and development of uremia (Bogin *et al.*, 1994).

The present study demonstrated that urine GGT and lactate dehydrogenase activities were significantly decreased in groups given N-acetylcysteine and/or vitamin E prior to gentamicin injection.

The decline observed in kidney gamma glutamyl transferase enzyme post CCL4 administration is an indication of impaired GSH synthesis. GGT enzyme is the first enzyme in the degradation of GSH to its constituent of amino acids which are introduced in the resynthesis of GSH to replenish the tissue stores (McLennan *et al.*, 1991 and Moawad, 2007).

Nephrotoxicity induced by cadmium quickly progressed as indicated by marked elevation in urinary LDH activity and protein excretion, NAC is very effective in protection against this toxicity (Shaikh *et al.*, 1999).

Our histopathological results revealed that kidney of rats injected with gentamicin showed tubulointerstitial nephritis as well as interluminal eosinophilic proteinaceous cast. NAC could protect the kidney against this damage when given prior to gentamicin injection, while N-acetylcystein with vitamin E showed marked improvement in texture of tubules and glomeruli.

Derakhshanfar *et al.* (2007) reported that after intramuscular administration for up to ten days; gentamicin (80 mg/kg b.wt) alone caused a significant reduction in glomerular filtration rate (GFR), glomerular changes and secondary tubular casts. They also showed that vitamin E could protect renal cells from this damage.

On the basis of the relationship between acute and long term damage, NAC would presumably have a protective effect on the chronic nephrotoxicity, so it is important for the protective agents to exist in renal tissue before damage occurs (Karimi *et al.*, 2005).

Conclusion:

In conclusion, the toxic effects and degree of renal failure induced by gentamicin were decreased by N-acetylcysteine and/or vitamin E. These compounds partially corrected the changes in enzymes activities and other parameters also protected kidney from tubular damage.

Conflict of Interest:

The authors have no potential conflicts of interest that are directly relevant to the contents of this article.

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