



## Biochemical and Oxidative Alterations Induced by Acute Amikacin Toxicity in Albino Wistar Rats

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Received: 09 Jan, 2018

Revised: 20 April, 2018

Accepted: 26 April, 2018

### ABSTRACT

Aminoglycoside antibiotics have long been used in antibacterial therapy. Despite their beneficial effects, aminoglycosides have considerable nephrotoxic and ototoxic side effects. So, present study was done to evaluate biochemical and oxidative changes in amikacin induced nephrotoxicity in experimental rats. A total of 24 rats were equally divided into four groups which were: Gr 1 injected with saline, Gr 2 injected with AK @ 50 mg/kg bw, Gr 3 injected with AK @ 100mg/kg bw and Gr 4 injected with AK @ 200mg/kg bw, respectively. Blood sampling was done on three days 0, 5<sup>th</sup> and 10<sup>th</sup> day of experiment. Blood samples were evaluated for BUN, creatinine and uric acid. Blood glutathione and MDA as an antioxidant biomarker was evaluated on 10<sup>th</sup> day of experiment. The plasma BUN, creatinine and uric acid levels increased significantly in all the groups in a dose and time dependent manner. Amikacin also increased MDA and decreased GSH levels in a dose dependent manner in all the groups compared to control group. It was concluded that amikacin induces the nephrotoxicity characterised by elevated levels of BUN, creatinine and uric acid and oxidative stress through elevating levels of MDA and decreasing levels of GSH.

**Keywords:** Amikacin, Biochemical, Oxidative stress, Nephrotoxicity

Aminoglycoside antibiotics are employed clinically because of their potent bactericidal activities, less bacterial resistance, post-antibiotic effects and low cost (Balakumar *et al.*, 2010). Aminoglycosides have long been reported as one of the commonest causes of drug induced nephrotoxicity (Walker and Duggin, 1988., Leclercq and Tulkens, 1999). Recent literatures demonstrated evidences of amikacin nephrotoxicity in different ways of its administration with different doses in rats. Nephrotoxicity is a major clinical complication of aminoglycoside antibiotics, as these are not metabolized in the body, and the most of injected dose is excreted in the urine, whereas a fraction accumulates in the renal proximal tubules cells, where the concentration of aminoglycosides is several times higher than that in plasma. The concentrated accumulation of aminoglycosides in the proximal tubules cells is associated with its nephrotoxic effects. Aminoglycosides administration is also reported to induce apoptosis (Klemens *et al.*, 2003), free radical

generation (Leclercq and Tulkens, 1999) and another major adverse effect (Gilbert *et al.*, 2000). Free radicals play an important role in drug-induced damage to the kidney failure and other organs (Cantin and woods, 1993). The purpose of present study was to evaluate effect of amikacin toxicity on biochemical and oxidative stress parameters in male albino rats.

### MATERIALS AND METHODS

Twenty four healthy adult albino Wistar rats of either sex, weighing approximately 150-200gm were used for study. The rats were randomly divided into four groups viz. Group 1 (control) and three toxic groups (Group 2, 3 and 4) each comprising six animals. The rats of control group (Gr 1) received potable water whereas nephrotoxic groups Gr 2, Gr 3 and Gr 4 were given amikacin intraperitoneally (IP) @ 50, 100 and 200 mg/kg body weight, respectively for

10 days. The experimental animals were closely observed for development of any clinical signs suggestive of nephrotoxicity throughout the entire period of experiment.

Samples for laboratory examinations were collected from rats of different groups at different intervals during the course of experiment. Blood samples from individual rat were collected in vials on day 0, 5 and 10. Blood samples were subjected to biochemical analysis for Blood urea nitrogen (Marsh *et al.*, 1965), Creatinine (Bonses and Taussky, 1945) and Uric acid (Newman and Price, 1994). Oxidative stress parameters such as lipid peroxidation (Rehman, 1984) and blood glutathione (Beutler, 1975) were estimated using hemolysate and whole blood, respectively on 10<sup>th</sup> day of experiment.

### Statistical analysis

The data were expressed as mean±SE. Standard error of mean and p values were used to determine any significant difference among different treatment groups using two-way analysis of variance (ANOVA) following standard protocol (Snedecor and Cochran, 1994).

## RESULTS AND DISCUSSION

Nephrotoxicity was induced in Wistar albino rats (Gr 2, Gr 3 and Gr 4) by intraperitoneal injection of amikacin sulphate @ 50 mg/kg (Gr 2), 100 mg/kg (Gr 3) and 200 mg/kg (Gr 4). Clinical signs, blood biochemical indices and oxidative parameters were estimated to evaluate the effect of amikacin on kidneys.

Plasma urea nitrogen (BUN) values recorded significant ( $P<0.05$ ) elevation from day 5 onwards in rats of Gr 2, Gr 3 and Gr 4 receiving amikacin than those kept as healthy control (Gr 1). However, highest value ( $79.50\pm5.84$ ) of BUN was found in rats of Gr 4 receiving highest dose of amikacin on day 10 (Table 1). Significantly ( $P<0.05$ ) higher value of plasma creatinine (mg/dl) was recorded from day 5 onwards in rats of Gr 2, Gr 3 and Gr 4 than the rats of healthy control group (Gr 1). The highest value ( $4.07\pm0.12$ ) of creatinine was estimated in rats of Gr 4 as compared to Gr 2 ( $2.21\pm1.07$ ) and Gr 3 ( $3.30\pm0.10$ ) on day 10 (Table 1). There was a dose dependent increase in BUN and creatinine levels in the rats of different groups receiving different doses of amikacin with significantly ( $P<0.05$ ) higher values noted in rats of Gr 4.

**Table 1:** Effect of different doses of amikacin on BUN (mg/dl) and Creatinine (mg/dl) level of rat

Sl. No.	BUN		
	Days after treatment		
	0 Day	5 Day	10 Day
Gr 1	$10.83\pm0.70^{Aa}$	$11.17\pm0.80^{Aa}$	$11.17\pm0.47^{Aa}$
Gr 2	$11.83\pm0.47^{Aa}$	$20.83\pm1.13^{Bb}$	$32.33\pm1.02^{Bc}$
Gr 3	$12.33\pm0.49^{Aa}$	$31.5\pm2.5^{Cb}$	$68.33\pm2.7^{Cc}$
Gr 4	$11.83\pm0.47^{Aa}$	$41.83\pm1.66^{Db}$	$79.50\pm5.84^{Dc}$
Sl. No.	Creatinine		
	Days after treatment		
	0 Day	5 Day	10 Day
Gr 1	$1.32\pm0.01^{Aa}$	$1.30\pm0.01^{Aa}$	$1.28\pm0.01^{Aa}$
Gr 2	$1.35\pm0.01^{Aa}$	$1.87\pm0.03^{Bb}$	$2.21\pm1.07^{Bc}$
Gr 3	$1.3\pm0.01^{Aa}$	$2.13\pm0.06^{Cb}$	$3.30\pm0.10^{Cc}$
Gr 4	$1.26\pm0.03^{Aa}$	$3.30\pm0.06^{Db}$	$4.07\pm0.12^{Dc}$

Mean±SE bearing different superscript (capital letters) vary significantly  $p<0.05$  between the groups while as Mean±SE bearing different superscript (small letters) vary significantly  $p<0.05$  within the group.

The plasma uric acid concentration in rats of Gr 2, Gr 3 and Gr 4 showed significant ( $P<0.05$ ) increase from day 5 onward as compared to corresponding values noted in healthy rats (Table 2). The highest value ( $7.50\pm0.15$ ) of uric acid was estimated in rats of Gr 4 as compared to Gr 2 ( $4.81\pm0.31$ ) and Gr 3 ( $6.10\pm0.31$ ) on day 10.

**Table 2:** Effect of different doses of amikacin on uric acid (mg/dl) level in rats

Sl. No.	Days after treatment		
	Uric Acid		
	0 Day	5 Day	10 Day
Gr 1	$3.14\pm0.02^{Aa}$	$3.15\pm0.03^{Aa}$	$3.15\pm0.02^{Aa}$
Gr 2	$3.33\pm0.11^{Aa}$	$3.96\pm0.01^{Bb}$	$4.81\pm0.31^{Bc}$
Gr 3	$3.33\pm0.06^{Aa}$	$4.31\pm0.08^{Bb}$	$6.10\pm0.31^{Cc}$
Gr 4	$3.32\pm0.06^{Aa}$	$6.06\pm0.14^{Cb}$	$7.50\pm0.15^{Dc}$

Mean±SE bearing different superscript (capital letters) vary significantly  $p<0.05$  between the groups while as Mean±SE bearing different superscript (small letters) vary significantly  $p<0.05$  within the groups

The results on the effect of intraperitoneal administration of different doses of amikacin once daily for 10 days on lipid peroxidation are shown in (Table 3). The peroxidation of membranes of erythrocytes expressed in terms of MDA levels were  $3.24\pm0.54$  MDA produced /mg

of Hb/ h in control animals. Amikacin at different doses induced significant ( $P<0.05$ ) peroxidation of membranes of erythrocytes in all groups in a dose dependent manner. A significant ( $P<0.05$ ) increase in lipid peroxidation was found on day 10<sup>th</sup> in amikacin treated rats as compared rats of control group (Gr 1).Where as blood glutathione (GSH) showed a decreasing trend in all groups in a dose dependent manner with lowest value in Gr 4 ( $23.64\pm1.47$  n mol/ml) as compared to normal control ( $56.73\pm0.82$  n mol/ml)) as shown in table 3.

**Table 3:** Effect of different doses of amikacin on lipid peroxidation (n mole MDA formed/ml erythrocytes or g tissue) and blood glutathione (GSH) (n mol/ml) in rats

Group No.	MDA	GSH
Gr 1	$3.45\pm0.54^A$	$56.73\pm0.82^A$
Gr2	$6.15\pm0.41^B$	$41.76\pm4.08^B$
Gr3	$7.92\pm0.56^{BC}$	$30.50\pm2.32^C$
Gr4	$9.05\pm0.91^{CD}$	$23.64\pm1.47^D$

Values given are mean  $\pm$  SE of the results obtained from 6 animals; Means with at least one common superscript do not differ significantly at 5% ( $P<0.05$ ) level of significance.

Rats of different experimental groups (Gr 2 to Gr 4) treated with different doses of amikacin showed signs of varying degree of inappetance, rough hair coat, polyuria and polydipsia. The typical clinical manifestation of aminoglycoside toxicity is nonoliguric or even polyuric renal excretion dysfunction (Lopez- Novoa, *et al.*, 2011). Progression to oliguric or anuric renal failure is infrequent, and recovery upon drug discontinuation is most often observed. High doses of aminoglycosides (40 mg/kg or more) are necessary in animals to rapidly induce extended cortical necrosis and overt renal dysfunction (Laurent *et al.*, 1982).

The concentration of urea nitrogen and creatinine in plasma increased in rats of Gr 2, Gr 3 and Gr 4 receiving amikacin injection @ 50 mg/kg, 100 mg/kg, 200 mg/kg and 400 mg/kg bw respectively from day 5 onwards. The increasing trend was more pronounced in high dose group than low dose groups. The present findings were in accordance with the earlier observations (Mehmet *et al.*, 2009; Mohamed *et al.*, 2010; Aya *et al.*, 2016 and Naseer *et al.*, 2014). Nephrotoxicity induced by aminoglycosides is a complex phenomenon characterized by an increase in

BUN, serum creatinine levels and severe proximal renal tubular necrosis followed by renal failure (Al-Majed *et al.*, 2002). Nagai *et al.* (2001) reported that amikacin accumulated most abundantly in the renal cortex, whereas amikacin was not detected in the renal papilla, brain, lung, and liver. The reduction in glomerular filtration rate, which is indicated by the increase in serum creatinine and BUN levels when a marked renal parenchymal injury occurs (Erdem *et al.*, 2000).

Amikacin treated rats showed significantly higher concentration of plasma uric acid. Maximum concentration was observed in the highest dose group. Association of increase in uric acid with renal dysfunction and amikacin-induced nephrotoxicity is suggested to be a complex phenomenon characterized by an increase in serum uric acid levels and severe proximal renal tubular necrosis followed by renal failure (Aya *et al.*, 2016).

The elevation in MDA level was attributed to oxidative stress and free radical generation through lipid peroxidation. This was in agreement with the results reported by Bert and Dev, 2006; Bulent *et al.*, 2006; Atef *et al.*, 2010. Overproduction of free radicals induces an increase in lipid peroxidation (MDA) by destroying unsaturated fatty acids in the cell membrane and cause decrease in endogenous antioxidants such as glutathione (GSH) in renal tissue (Kaynar *et al.*, 2007).

## CONCLUSION

It may be concluded that amikacin causes marked alteration in biochemical and oxidative parameters that are markers of stress induced nephrotoxicity and hence causes nephrotoxicity.

## ACKNOWLEDGMENTS

Authors are thankful to the ICAR, New Delhi for financial support for conducting research work.

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