doi.org/10.36721/PJPS.2021.34.3.REG.869-874.1

Protective role of salicylic acid in gentamicin induced nephrotoxicity

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Abstract: Salicylic acid, a phenolic compound, found in plants, possesses free radical scavenging and iron chelation properties. The present study is designed to study the antioxidant effect of salicylic acid in gentamicin induced nephrotoxicity in rabbits. For this purpose twenty four male albino rabbits were divided into 4 groups (n=6); control group, healthy untreated rabbits, gentamicin group, received only gentamicin (80mg/kg), gentamicin + salicylic acid group, received gentamicin (80mg/kg) + salicylic acid (80mg/kg) and salicylic acid group, received only salicylic acid (80mg/kg) via intra peritoneal route for 21 consecutive days. Biochemical evaluation was carried out by assessment of body weights and by estimating renal function tests (plasma urea, plasma creatinine and plasma uric acid), tissue antioxidant enzymes (catalase, SOD) and MDA level. Gentamicin induction resulted in decreased body weights, increased plasma urea, plasma creatinine, plasma uric acid, tissue MDA level and decreased tissue SOD and tissue catalase activity in gentamicin treated group which was restored by supplementation with salicylic acid in gentamicin + salicylic acid group. Our data suggests that supplementation of salicylic acid can be useful in reducing gentamicin induced nephrotoxicity in rabbits.

Keywords: Gentamicin, salicylic acid, plasma urea, plasma creatinine, plasma uric acid antioxidant enzymes.

INTRODUCTION

Nephrotoxicity induced by gentamicin (GEN is a complex phenomenon characterized by an increase in blood urea and serum creatinine concentration, and severe proximal renal tubular necrosis followed by deterioration and renal failure (Pavle *et al*., 2017). Although the pathogenesis is still not well understood, the toxicity of GEN in the kidney seems to relate to the generation of destructive reactive oxygen species (ROS) in these cells (Reiter *et al*., 2002). ROS have been implicated in a wide range of biological functions, but they can express both beneficial and highly toxic effects on cellular homeostasis (Mates, 2000). A large body of in vivo and in vitro evidence indicates that ROS are important mediators of GEN-induced nephrotoxicity (Kopple *et al*., 2002). ROS have been proposed as a causative agent of cell death in many different pathological states as well as, in glomerular disease (Smetana *et al*., 1988), in renal ischemia and reperfusion injury (Longoni *et al*., 2002) and in various models of toxic renal failure (Piotrowski *et al*., 1996).

Gentamicin increases generation of reactive oxygen species (ROS) such as super oxide anions, (Ehsani *et al*., 2017) hydroxyl radicals, hydrogen peroxide, and reactive nitrogen species in the kidney (Walker *et al*., 1999). Gentamicin induced kidney damage is linked with lipidperoxidation (Ali *et al*., 2002) and protein oxidation in the renal cortex (Serner *et al*., 2002). Gentamicin reduces activity of renal antioxidant enzymes like superoxide dismutase (SOD), catalase, glutathione peroxidase (GP), and glutathione (Polat *et al*., 2006; Karadeniz *et al*., 2008).

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Phenolic compounds from dietary plants are known to be good scavengers of reactive oxygen species. In the past few decades, a considerable and consistent amount of evidence has demonstrated that SA has antioxidant properties (Baltazar *et al*., 2011, Colantoni *et al*., 1998), though the mechanisms underlying these effects remain unclear. Firstly, it has been reported that salicylates comprises of free radical-scavenging and iron chelation properties (Aruoma *et al*., 1988). Also, it has been demonstrated that salicylate effectively protects against gentamicin-induced hearing loss in guinea pigs (Sha *et al*., 1999). Thus, the present study proves anti-oxidant properties of Salicylic acid in gentamicin induced nephrotoxicity.

MATERIALS AND METHODS

Twenty four male albino rabbits (1200-1500g body weight) were purchased from local market (Karachi, Pakistan) for the study. Animals were acclimatized to the laboratory conditions one week before the start of experiment and caged in a quite temperature controlled room (23±4 °C). Animals had free access to water and diet. The experiments were conducted with ethical guidelines of institutional ERB (Ethical Review Board) and internationally accepted principles for laboratory use and care in animal research (Health research extension Act of 1985).

Study Design

After a quarantine period of one week, twenty four rabbits were divided randomly into four groups, each consisting of 6 animals (n=6).

Control group: Healthy untreated animals.

Gentamicin group: Received only gentamicin (80mg/kg)
Gentamicin + salicylic acid group: Received gentamicin (80mg/kg) + salicylic acid (80mg/kg)
Salicylic acid group: Received only salicylic acid (80mg/kg).

At the end of twenty-one days experimental period, rabbits were weighed and sacrificed after twenty four hours of last dose of gentamicin and salicylic acid administration. Blood was collected in lithium heparin coated tubes then was centrifuged to obtain plasma for estimation of plasma urea, creatinine and uric acid. Kidneys were excised, trimmed of connective tissues, rinsed with saline to eliminate blood contamination dried by blotting with filter paper and weighed. Biochemical assessments were made by estimating renal function tests and renal antioxidant enzymes status.

Preparation of Post mitochondrial supernatant (PMS)

Post mitochondrial supernatant was prepared by chopping one kidney into small pieces then add 10ml of chilled 5mM potassium phosphate buffer (pH 7.8) and made homogenate by using a homogenizer. The homogenates were centrifuged at 800g for five minutes at 4ºC to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500g for 20 minutes at 4ºC to get post mitochondrial supernatant which was used to assay tissue SOD, Catalase and MDA activity.

Estimation of Malonyldialdehyde (MDA)

The malonyldialdehyde (MDA) content, a measure of lipid peroxidation was assayed in the form of thiobarbituric acid reacting substances (TBARS) (Okhawa *et al*, 1979). The reaction mixture consisted of 0.2mL of 8.1% sodium dodecylesulphate, 1. 5mL of 20% acetic acid solution adjeusted to pH=3.5 with sodium hydroxide and 1.5mL of 0.8% aqueous solution of thiobarbituric acid was added to 0.2mL of 10% (w/v) of homogenate. The mixture was brought up to 4.0mL with distilled water and heated at 95°C for 60 minutes. After cooling with tap water, 1.0mL distilled water and 5.0mL of the mixture of n- butanol & pyridine (15:1 v/v) was added and centrifuged. The organic layer was taken out and its absorbance was measured at 532 nm on schimadzu spectrophotometer UN 120-01 and compared with those obtained from MDA standards. The concentration values were calculated from absorption measurements as standard absorption in nM/g tissue (Okhawa *et al.*, 1979).

Assessment of renal functions

Plasma samples were assayed for urea and creatinine. Urea was estimated spectrophotometry by the Oxime method (Butler *et al*., 1981). Creatinine was estimated spectrophotometry by the Jeff's method (Spierto *et al*., 1979). Uric acid was estimated spectrophotometry using kit method.

Assessment of Antioxidant Status

Estimation of Catalase

Catalase activity was assayed by the method of Sinha *et al*, 1972, in a clean glass test tube, the assay mixture consisted of 1.96mL of phosphate buffer (0.01M, pH= 7.0), 1.0mL of hydrogen peroxide (0.2M) and 0.04mL (10mL of 2 m L of dichromate acetic acid reagent (5% of 50mL dichromic acid + 150mL ofglacial acetic acid) was added in 1mL of reaction mixture, boiled for 10min, cooled. Changes in absorbance were recorded at 570nm on Schimadzu-spectrophotometer UV 120-01. The concentration values were calculated from absorption measurements as standard absorption in mM/g tissue (Sinha, 1972).

Estimation of Superoxide Dismutease (SOD)

Levels of SOD in the cell free supernatant were measured by the method of Kono *et al*, 1978. Briefly, 1.3mL of solution A (0.1mM EDTA containing 50mM Na2CO3 pH=10.0), 0.5ml of solution B (90µM NBT-nitrobluetetr-azoliumdye) and 0.1ml of solution C (0.6% triton-100 insolution A), 0.1ml of solution D (20mMHydroxylamine hydrochloride, pH= 6.0) were mixed and rate of NBT reduction was recorded for one minute at 560 nm on Schimadzu spectrophotometer UV120-01. 0.1 ml of the supernatant was added to the test and reference cuvettes, which do not contain solution D. Finally, the % inhibition in the rate of reduction of NBT was recorded as described above in U/g tissue. One enzyme unit was expressed as inverse of the amount of protein (mg) required inhibiting the reduction rate by 50% in one minute. The activity was calculated using the % inhibition in gram of tissue and expressed in Unit/g tissue (Kono *et al.,* 1978).

STATISTICAL ANALYSIS

The presented data is expressed as mean ± standard deviation and was analyzed by one way ANOVA using SPSS version 22 to determine the difference among the mean values of experimental groups. $P<0.05$ was considered as significant.

RESULTS

The effect of gentamicin and salicylic acid induction on body weight of experimental rabbits

Table 1, fig. 1 showed a significant weight loss in gentamicin induced group (8.70±0.78) as compared to control group weight gain (8.97±1.76) where as in GM+SA treated group weight loss (5.08±0.53) was markedly less than that of gentamicin induced group, confirms the weight gaining property of salicylic acid as also observed in salicylic acid group (3.90±0.58).

The effect of gentamicin and salicylic acid induction on plasma urea in experimental rabbits

Gentamicin induction resulted in significantly increased level of plasma urea (41.00±1.26) in GM-treated renal

Table 1: Change in body weight of different groups of rabbits over 21 days study period

Group	Initial Weight (gm)	Final Weight (gm)	Weight Gain (%)	Weight Loss (%)
Control	1300.83	1416.17	8.97 ± 1.76	--
Gentamicin	1316.67	1202.33	--	8.70±0.78
GM+SA	1297.33	1231.50	--	5.08±0.53
Salicylic Acid	1289.17	1341.00	3.90 ± 0.58	--

Table 2: The effect of gentamicin and salicylic acid induction on plasma urea, plasma creatinine and plasma uric acid levels in experimental rabbits

Parameters	Control	Gentamicin	GM+SA	Salicylic Acid
Plasma Urea (mg/dl)	28.17±1.17	41.00±1.26 [(a)]	27.83±1.33 [(b)]	31.50±2.26
Plasma Creatinine (mg/dl)	0.95±0.19	3.17±0.08 [(a)]	0.87±0.05 [(b)]	0.88±0.12
Plasma Uric Acid (mg/dl)	1.53±0.03	2.35±0.03 [(a)]	1.54±0.02 [(b)]	1.55±0.03

Table 3: Effect of gentamicin and salicylic acid induction on renal MDA level and SOD & CAT activities in experimental rabbits

Parameters	Control	Gentamicin	GM+SA	Salicylic Acid
MDA (nmol/gtissue)	6.09±0.03	7.59±0.04 [(a)]	6.26±0.05 [(b)]	6.06±0.03
CAT (unit/ml)	34.88±0.63	23.35±0.69 [(a)]	37.00±0.93 [(b)]	36.92±1.48
SOD (U/gm tissue)	17.65±1.10	10.68±1.40 [(a)]	17.45±1.34 [(b)]	16.75±2.00

Data are shown as means ± SD, (a) P<0.05 versus control group. (b) P<0.05 versus Gentamicin group

injury group as compared to the control group (28.17±1.17). Salicylic acid administration markedly reduced plasma urea level in GM + SA group (27.83±1.33) as compared to GM-treated group.

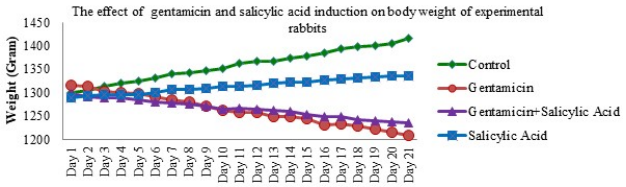

Fig. 1: The effect of gentamicin and salicylic acid induction on body weight of experimental rabbits

The effect of gentamicin and salicylic acid induction on plasma Creatinine level in experimental rabbits

Plasma creatinine level was significantly increased in the GM-treated (3.17±0.08) renal injury group as compared to the control group (0.95±0.19). Salicylic acid administration restored creatinine level in GM + SA group (0.87±0.05) as compared to GM-treated group. In salicylic acid treated group, plasma creatinine level was found almost similar in the control and GM+ SA groups (0.88±0.12) (table 2, fig. 3).

The effect of gentamicin and salicylic acid induction on plasma uric acid of experimental rabbits

Plasma uric acid level was significantly increased (2.35±0.03) in the GM-treated renal injury group as compared to the control group (1.53±0.03). Salicylic acid administration restored it in GM+ SA group (1.54 ±0.02) as compared to GM-treated group whereas in alone salicylic acid group, plasma uric acid was found almost similar to that of control group (table 2, fig. 3).

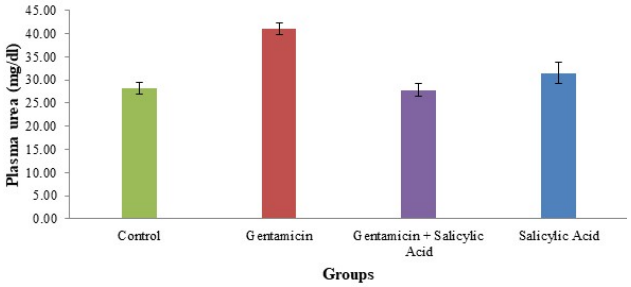

Fig. 2: The effect of gentamicin and salicylic acid induction on plasma Urea level in experimental rabbits

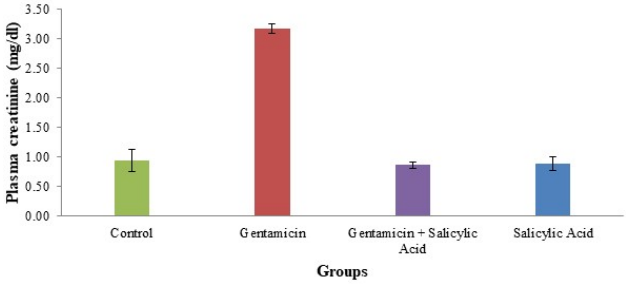

Fig. 3: The effect of gentamicin and salicylic acid induction on plasma Creatinine level of experimental rabbits

The effect of gentamicin and salicylic acid induction on tissue MDA level in experimental rabbits

Tissue MDA was significantly increased (7.59±0.04) in the GM-treated renal injury group, when compared to the control group (6.09±0.03).The elevation of MDA level

induced by GM were completely prevented by SA administrations in GM + SA group (6.26±0.05). The MDA contents were found similar in the control and SA groups (6.09±0.03, 6.06±0.03).(table 3, fig. 5).

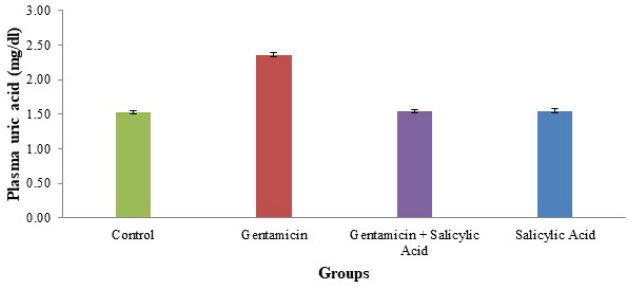

Fig. 4: The effect of gentamicin and salicylic acid induction on plasma Urea level of experimental rabbits

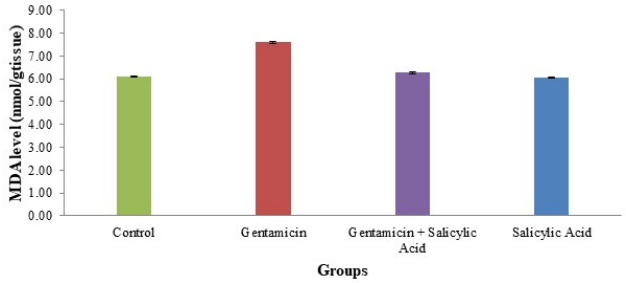

Fig. 5: The effect of gentamicin and salicylic acid induction on tissue MDA level in experimental rabbits

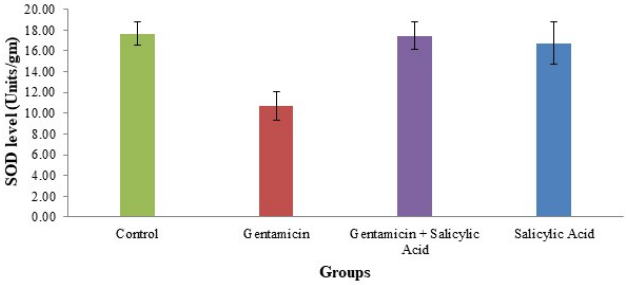

Fig. 6: The effect of gentamicin and salicylic acid induction on superoxide dismutase activity in experimental rabbits

The effect of gentamicin and salicylic acid induction on tissue superoxide dismutase activity in experimental rabbits

Tissue SOD activity was markedly decreased (12.15 ±2.68) in the GM-treated renal injury group when compared to the control group (15.50±2.06). The reduction induced by GM was completely prevented by SA administrations in GM + SA group (17.45±1.34), In alone salicylic acid group tissue SOD activity was almost near to normal (16.75±2.00) as compared to control group. (table 3, fig. 6).

The effect of gentamicin and salicylic acid induction on tissue catalase activity in experimental rabbits

Tissue Catalase was significantly decreased (23.35±0.69Units/gm) in the GM-treated renal injury group, when compared to the control group (34.88±0.63Units/gm). The decreases induced by GM were completely prevented by SA administrations in GM + SA group (37.00±0.93). The Catalase contents were found similar in the control and SA groups. (table 3. fig. 7).

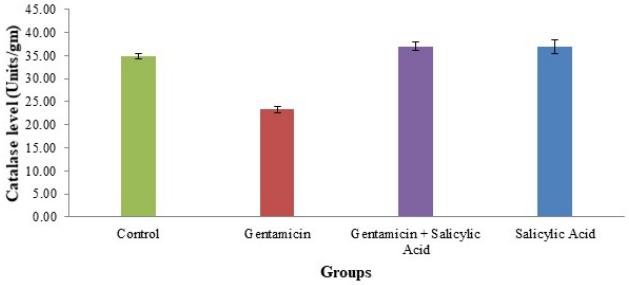

Fig. 7: The effect of gentamicin and salicylic acid induction on catalase activity in experimental rabbits

DISCUSSION

Aminoglycoside antibiotics play an integral role in antimicrobial chemotherapy. Unfortunately, these drugs are known to cause nephrotoxicity in man and in experimental animals. Gentamicin as one of the aminoglycoside antibiotics exerts its toxic effect directly on the proximal tubule causing acute tubular necrosis. Induction of gentamicin causes acute renal failure, continuous body weight loss, associated with reduced food intake, following development of condition of acidosis with increased rate of catabolism (Venkatesha and Veeru; 2019). Present study also showed a significant loss of body mass after twenty-one days of gentamicin induction (table 1, fig. 1) which was successfully regained after supplementation with salicylic acid. The toxic effect induced by gentamicin administration was found to be mediated by generating reactive oxygen species (ROS) (Sha SH *et al*., 1999). As it is indicated by increased plasma urea, creatinine and uric acid level after gentamicin induction that was significantly restored through free radical scavenging properties of salicylic acid (table 2, figs. 2-4).

A relationship between oxidative stress and nephrotoxicity has been well demonstrated in many experimental animal models (Somova *et al*., 2003). Gentamicin-injected rabbits showed a significant increase in lipid peroxidation products as malondialdehyde (MDA) suggesting that the involvement of oxidative stress, which has been reported, while administration of Salicylic acid with GM tended to normalize the level of MDA (table 3, fig. 5). A role of lipid peroxidation in gentamicin-induced nephrotoxicity has also been described in previous studies. Injection of gentamicin at a nephrotoxic dose induced a marked renal failure, characterized by significant increase in lipid peroxidation. Gentamicin injection at a dose of (80mg/kg b.wt.) induced kidney damage as indicated by significant increase in MDA (El-

Ashmawy *et al*., 2006). Meanwhile, treatment of animals with hydroxyl radical scavenger salicylic acid has been shown to protect against gentamicin-induced acute renal failure (Kumar *et al*., 2000). The nephroprotective effect of salicylic acid may be due to salicylic acid reduce lipid-peroxidation by direct antioxidant effect (Sailaja and Krishna 2017).

In the current study, GM induced oxidative stress which results in decrease in antioxidant enzymes like catalase and superoxide dismutase (SOD) (table 3, fig. 6, 7). There are some experimental data suggesting that nephrotoxic drugs may also change levels of MDA, CAT & SOD (Hanieh *et al*., 2018) which are commonly used to monitor the development and extent of renal tubular damage due to oxidative stress. Thus, the preventive effect of salicylic acid induce decrease in the activity of superoxide dismutase (SOD) and CAT could be contributed to the restoration of markers of renal tubular injury. It seems reasonable to assume that salicylic acid is able to suppress nephrotoxicity in kidney.

CONCLUSION

In conclusion, the gentamicin induced nephrotoxicity may be related with oxidative damage. Co-administration of salicylic acid decreased the harmful effects of gentamicin both by inhibiting free-radical formation and by restoration of the antioxidant systems. Further investigations on the mechanism of action of salicylic acid are required and may have a considerable impact on future clinical treatments of patients with renal disorders.

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