

Effect of salicylic acid supplementation on blood glucose, lipid profile and electrolyte homeostasis in gentamicin sulphate induced nephrotoxicity

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Abstract: This study was designed to investigate effect of salicylic acid supplementation in gentamicin- induced nephrotoxicity. 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) through intra peritoneal route for 21 consecutive days. Biochemical evaluation was carried out by assessment of body weights and by estimating plasma glucose, lipid profile and electrolyte homeostasis. Gentamicin sulphate induction resulted in increased plasma glucose, plasma TG, plasma cholesterol, plasma LDL, and plasma sodium and in decreased plasma HDL and plasma potassium with significant reduction in body weights in GS-treated group, which were restored by supplementation with salicylic acid in GS+SA treated group. Therefore, these findings confirm the protective role of salicylic acid in gentamicin- induced nephrotoxicity in rabbits.

Keywords: Gentamicin sulphate, salicylic acid, plasma glucose, lipid profile, electrolytes.

INTRODUCTION

Initially, gentamicin was extracted from *Micromonospora purpurea*, gram-positive bacteria extensively found in water and soil, and was recognized to have efficacy in the treatment of a variety of gram-negative bacterial infections (Cristina *et al.*, 2015). Gentamicin suppresses bacterial protein formation by binding to 30S ribosomal subunit and hinders the production of initiation complex with mRNA and also induces the incidence of misreading of the mRNA message, directing to the formation of defective proteins that provide bactericidal action (Xinshuai Zhang *et al.*, 2020). Gentamicin, an aminoglycoside, consists of two amino sugars coupled with hexose nucleus i.e. 2-deoxystreptamine through a glycosidic linkage. Gentamicin is polycationic at physiological pH that causes it freely water soluble (Eljaaly *et al.*, 2019). It undergoes small metabolism and is finely distributed in an individuals' body tissues. Gentamicin goes through limited re-absorption by proximal tubular cells by adsorptive endocytosis that results in the union of endocytic vacuoles with lysosomes where the drug accumulates (AGG Coutinho., 2018). This accretion causes the process of lysosomal phospholipidosis, resulting in tubular necrosis, which is the main pathological process leading to renal toxicity. Gentamicin is not proposed to be used orally as it is not absorbed at all significant degree from the small intestine. It is injected intravenously, intramuscularly or topically to

deal bacterial infections. It is totally excluded as unchanged manner by glomerular filtration by urine (Pitchai *et al.*, 2010).

In the past, much interest has been laid on the function of naturally occurring dietary substances for the control as well as management of a variety of chronic diseases. One such compound is salicylic acid (SA), which has been utilized ever since ancient times to grant pain relief as well as treat inflammatory situation. Salicylic acid is a phenolic compound found in plants, where it performs a vital role in the development of local as well as systemic resistance to pathogen infection. Humans, in addition to animals, obtain SA primarily from daily foods, fruits, along with vegetables. Mounting proof demonstrates that applied SA may counteract oxidative damage induced through adverse conditions in animals, although the mechanisms fundamental to these effects stay unclear. It has been reported that SA consists of free radical-scavenging as well as iron chelation properties. Phenolic compounds from dietary plants are recognized to be excellent scavengers of reactive oxygen species. The restorative effect of SA on the kidney markers might be attributed to its antioxidant abilities as it has been observed that ROS might be involved in the impairment of glomerular filtration rate (GRF) (Pavle *et al.*, 2012).

MATERIALS AND METHODS

Twenty four male albino rabbits weighing about 1200gm to 1500gm were selected for the study. These rabbits were

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purchased from local market (Karachi, Pakistan) and were acclimatized to the laboratory conditions one week before the start of experiment and caged in a quite temperature controlled room ($23\pm 4^{\circ}\text{C}$). 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).

Experimental protocol

Rabbits were divided into four experimental groups, six rabbits in each group.

Control group: remained untreated

GS group: received gentamicin sulphate (80 mg/kg)

GS+SA group: received gentamicin sulphate (80mg/kg) + salicylic acid (80mg/kg)

SA group: received salicylic acid (80mg/kg)

At the end of twenty-one days experimental period, rabbits were weighed and sacrificed after twenty four hours of the last dose of Gentamicin & Salicylic acid administration. Blood was collected in lithium heparin coated tubes and then was centrifuged to obtain plasma for estimation of plasma glucose, triglycerides, cholesterol, LDL, HDL, plasma sodium and plasma potassium and was also processed for estimation of intraerythrocyte sodium and potassium.

Biochemical analysis

Analysis of Glucose and Lipid Profile

Plasma glucose and lipid profile were analyzed by Spinreact kit using micolab 300.

Estimation of plasma sodium and potassium

Plasma was diluted 1:100 with 0.1N HCl and was used for simultaneous determination of sodium and potassium. The emission intensities of standards and samples were recorded against the respective blank solutions. The emission intensities of sodium, potassium were recorded at 589 and 768nm, respectively.

Erythrocyte membrane preparation

The packed red cells extracted by centrifugation at 4°C , 450g for 15 minutes were resuspended and diluted in 25 volumes of 0.011 mol/L Tris-HCl buffer at pH 7.4. The hemolyzed cells were then centrifuged for 30 min at 12,000 rpm at 4°C and the membrane pellet was resuspended in 30 ml of 0.011 mol/L Tris-HCl buffer. This centrifugation step was repeated three times. The final concentration of the membrane suspension was ~ 4 mg protein/ml of Tris buffer. The membrane suspension was stored at -70°C until the assay was performed.

Estimations of Intra erythrocyte sodium and potassium

Heparinized blood was centrifuged and plasma was separated. Buffy coat was aspirated and discarded. Erythrocytes were washed three times at room

temperature by suspension in the magnesium chloride solution (112mmol/L), centrifugation of 4500 g at 4°C for 5 min and aspiration of the supernatant (Fortes and Starkey, 1977). The final supernatant was retained for the estimation of intraerythrocyte sodium and potassium concentration. Neither electrolyte was detectable in the final wash. Washed erythrocytes were then used for the estimation of intraerythrocytes sodium and potassium.

STATISTICAL ANALYSIS

The values were presented as means \pm SD of different groups. Differences between the mean values were estimated using one way ANOVA. The results were considered statistically significant when $*p<0.01$ and $**p<0.05$.

RESULTS

Effect of gentamicin and salicylic acid induction on body weight in experimental rabbits

Body weights of GS treated groups were significantly decreased as compared to that of the control group (GS: 1262.619 ± 33.479 $p<0.01$). The body weight of GS+SA treated group was significantly increased (SA: 1265.524 ± 19.423 $p<0.01$) as compared to that of the GS treated group. Similarly the body weights of SA group were markedly reduced (SA: 1313.952 ± 15.002 $p<0.01$) as compared to that of the control group (fig.1).

Effect of gentamicin and salicylic acid induction on plasma glucose concentration in experimental groups

Plasma glucose was significantly increased in GS treated group as compared to control (GS: 46.192 ± 9.969 $p<0.01$). Significant reduction in glucose level was observed in GS+SA treated group as compared to the GS treated group (GS+SA: 21.320 ± 10.645 ; $p<0.01$). However salicylic acid (SA) treated group showed a reduction in the concentration of plasma glucose as compared to control (SA: 17.587 ± 6.711 ; $p<0.01$) (table.1).

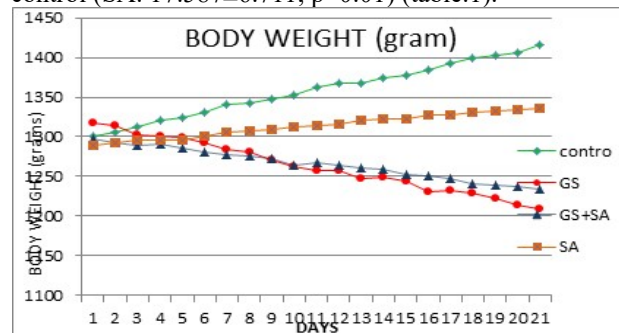


Fig.1: Effect of GS and SA induction on body weights of experimental groups

Effect of gentamicin and salicylic acid induction on plasma TG concentration in experimental groups

Plasma triglyceride was significantly increased in GS-treated group as compared to control (GS: 82.947 ± 10.21

Table 1: Effect of gentamicin and salicylic acid induction on plasma glucose and lipid profile in experimental groups.

Groups	Parameters				
	Glucose mg/dl	TG mg/dl	Cholesterol mg/dl	HDL mg/dl	LDL mg/dl
Control	28.797± 6.639	75.562±5.116	80.377±6.766	24.115± 2.029	56.265±4.741
GS	46.192±9.969*	82.947±10.21**	88.882±5.893**	22.598±1.027**	62.213±4.126**
GS+SA	21.320±10.645*	71.398±7.146**	84.200±3.98**	25.258±1.195**	58.933±2.793**
SA	17.587±6.711*	64.578±6.814**	75.343±3.426**	26.662±1.769**	52.735±2.399**

Table 2: Effect of gentamicin and salicylic acid induction on plasma Na⁺ and K⁺ in experimental groups.

Parameter	Experimental groups			
	Control	GS	GS+SA	SA
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Na ⁺ mmol/L	159.8±10.6	141.5 ± 3.2 *	163.1 ± 2.0 *	160.6 ± 4.4
K ⁺ mmol/L	3.875±1.821	5.25±2.986**	3.525±4.065**	3.25±2.802**
Intra-erythrocyte Na ⁺ mmol/L	14.7±1.3	18.3±1.9*	14.8±1.7*	8.7±0.5*
Intra-erythrocyte K ⁺ mmol/L	180.0±2.5	144.9±6.8*	174.2±4.9*	182.3±6.1*

Values are mean ± SD. Statistical significance and different from control and GS values and p-values of *p<0.01 and **p<0.05 are considered significant.

p<0.05). The level of TG in GS+SA group was reduced significantly as compared to GS- treated group (GS+SA: 71.398±7.146 p<0.05). While salicylic acid treated group significantly showed decreased level of TG as compared to control (SA: 64.578±6.814 p<0.05) (table.1).

Effect of gentamicin and salicylic acid induction on plasma cholesterol concentration in experimental groups

Plasma cholesterol was increased significantly in GS treated group as compared to control (GS: 88.882±5.893 p<0.05).The level of cholesterol was decreased in GS+SA treated group as compared to GS treated group (GS+SA 84.200±3.98 p<0.05). However, there was a significant reduction in plasma cholesterol in SA treated group as compared to control (SA.75.343±3.426 p<0.05) (table 1).

Effect of gentamicin and salicylic acid induction on plasma HDL in experimental groups

Plasma HDL was significantly decreased in GS treated group as compared to control (GS: 22.598±1.027; P<0.05). The level of plasma HDL was increased in GS+SA group as compared to GS- treated group (GS+SA: 25.258±1.195p<0.05), while in SA treated group plasma HDL markedly increased as compared to control (SA: 26.662±1.769p<0.05) (table-1).

Effect of gentamicin and salicylic acid induction on plasma LDL in experimental groups

Plasma LDL in GS- treated group was increased significantly as compared to control group (GS: 62.213 ± 4.126 p<0.05), and the level of LDL in GS+SA treated group was significantly decreased as compared to GS-treated group (GS+SA 58.933±2.793 p<0.05). Whereas in SA (salicylic acid) treated group, the level of LDL markedly decreased as compared to control (SA: 52.735 ± 2.399; p<0.05) (table 1).

Effect of gentamicin and salicylic acid induction on plasma Na⁺ concentration in experimental groups

Plasma sodium level was significantly decreased (141.5±3.2mmol/L) in the GM-treated renal injury group, when compared to the control group (159.8±10.6 mmol/L; P<0.01). The decreases induced by GM were completely prevented by SA administration (GM + SA group). The plasma sodium level was found similar in the SA and control groups (table 2).

Effect of gentamicin and salicylic acid induction on plasma k⁺ concentration in experimental groups

The level of plasma K⁺ was significantly increased in GS-treated group as compared to control (GS: 5.25±2.986; p<0.05). Salicylic acid administration in GS+SA group resulted in decreased concentration of plasma k⁺ as compared to GS group (GS+SA3.525±4.065; p<0.05),while SA- treated group showed slightly decreased level of plasma K⁺ as compared to control (SA: 3.25±2.802 p<0.05) (table. 2)

Effect of gentamicin and salicylic acid induction on intraerythrocyte Na⁺ concentration in experimental groups

Intra erythrocyte sodium level was significantly increased (18.3±1.9 mmol/L) in the GM-treated renal injury group, when compared to the control group (14.7±1.3 mmol/L; P<0.01). The increases induced by GM were completely prevented by SA administrations (GM + SA group). The intra erythrocyte sodium level was found reduced in the SA group as compared to that of control group (table 2).

Effect of gentamicin and salicylic acid induction on intraerythrocyte k⁺ concentration in experimental groups

Intra erythrocyte potassium level was significantly decreased (144.9±6.8mmol/L) in the GM-treated renal

injury group, when compared to the control group ($180.0 \pm 2.5 \text{ mmol/L}$; $P < 0.01$). The decreases induced by GM were completely prevented by SA administration (GM + SA group). The intra erythrocyte potassium level was found increased in the SA group as compared to that of control group (table 2).

DISCUSSION

Salicylic acid is a phenolic compound found in plants, where it performs a vital role in the development of local as well as systemic resistance to pathogen infection. Mounting proof demonstrates that applied SA may counteract oxidative damage induced through adverse conditions in animals, although the mechanisms fundamental to these effects stay unclear. It has been reported that SA consists of free radical-scavenging as well as iron chelation properties. The restorative effect of SA on the kidney markers might be attributed to its antioxidant abilities as it has been observed that ROS might be involved in the impairment of glomerular filtration rate (GRF) (Pavle *et al.*, 2012; Francisco., 2014). Body weight is an important indicator of adverse effects of antibiotics and it is considered as a determinant parameter of toxicity testing. The data indicate that the GS at a dose of 80mg/kg for 21 days decreases the body weight of test (GS) group. Ravindra Kumar reported the treatment of GS at a dose of 80mg/kg i.p in male albino rats reduces the body weight as compared to control (Ravindra., 2017). However, considerable weight gains observed in GS+SA group indicates that SA attenuates the effect of gentamicin on body weight of rabbits (Ravindra, 2017).

SA powerfully scavenges OH; the main confirmation for this activity is the capability of SA to go through aromatic hydroxylation through reacting with OH (Teresa *et al.*, 2011). The gentamicin sulphate treated rabbits significantly showed increase in plasma glucose concentration as compared to control group. This result is in agreement with Ramar Manikanda who reported that treatment of rats with gentamicin (100 mg/kg) increased the level of serum glucose as compared to control. However decreased blood glucose level in GS+SA group indicates salicylic acid scavenging property (Ramar., 2011). In normal conditions, glucose is freely filtered at the glomerulus; however, approximately all of it is reabsorbed by the proximal tubule. While this section of the nephron is damaged, glucose might appear in the urine. Such renal glycosuria has been observed in alliance with renal failure induced through some proximal tubular toxins (David., 1976; Sun *et al.*, 2012).

Gentamicin induction at a dose of 80 mg/kg body weight showed significant effects on lipid profile, the level of TG, cholesterol and LDL that were markedly increased in group II rabbits (gentamicin 80mg/kg body weight I.P), however, the level of HDL was slightly decreased as

compared to control group. Similarly, Umbreen reported that treatment of rats with gentamicin (80 mg/kg body weight I.P) increased the level of serum TG, cholesterol, and LDL and a decrease in the level of HDL as compared with control group, while the treatment of salicylic acid along with gentamicin in GS+SA group significantly reduced the levels of TG, cholesterol and LDL strongly and markedly increased the levels of HDL indicating that SA possesses free radical-scavenging as well as iron chelation properties (Umbreen 2017; Teresa *et al.*, 2011).

The exacerbated rise of lipid peroxidation through gentamicin impairs membrane lipids. The oppressive effect of gentamicin on the nonenzymatic antioxidants in addition to enzymatic antioxidants results in an excess in the production of reactive oxygen species, which not only deleteriously affects membrane lipids but also deteriorates proteins in addition to nucleic acids. The high levels of triglycerides might be due to the inhibition of 7 α -hydroxylase activity. The high concentration of LDL-C might be attributed to a down regulation in LDL receptors (Munira 2017).

The increase in LDL-c level might be explained by means of involvement of two enzymes, namely cholesterol ester hydrolase plus cholesterol ester synthetase. These enzymes regulate the cholesterol levels in the blood. For this reason, it is logical to suppose that the increase in plasma cholesterol is mediated during increased cholesterol turnover in addition to being influenced by the relative balance between cholesterol ester hydrolase as well as cholesterol ester synthetase activity. With increased esterifying activity (when cholesterol ester hydrolase: Cholesterol ester synthetase is lowered) cholesterol exists predominantly in its ester form (as in LDLc) thereby leading to the development of atherosclerosis (Munira 2017 and Mustad 1997).

Gentamicin administration (at a dose of 80mg/kg) resulted in decreased plasma Na^+ in GS treated group, while plasma K^+ was increased. Coskun reported that treatment of Gentamicin sulfate (80 mg/kg per day i.p.) in male Wistar rats markedly decreased plasma Na^+ and increased plasma K^+ (Coskun., 2007). However, salicylic acid at the same dose raised the plasma Na^+ and reduced plasma K^+ in GS+SA treated group indicating that SA consists of free radical-scavenging as well as iron chelation properties. It has been shown that aminoglycoside antibiotics have harmful effects to the kidneys as they form ROS and salicylic acid powerfully scavenging reactive oxygen species through its antioxidant properties.

CONCLUSION

According to current biochemical findings, supplementation with salicylic acid significantly reduced nephrotoxic effects of gentamicin sulphate induction.

Thus, it confirms the protective role of salicylic acid against gentamicin induced nephrotoxicity in rabbits.

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