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Salicylic acid protects gentamicin-induced hepatotoxicity: Study in rabbits

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Abstract: Gentamicin (GM) is a broadly used antibiotic against severe and life-threatening infections, but its efficacy is restricted by the development of liver toxicity. The present study was designed to evaluate the protective effect of salicylic acid (SA) in gentamicin-induced hepatotoxicity in rabbits. Gentamicin and salicylic acid were given at a dose of 80 mg/kg i.p for twenty days. For this purpose, 24 male albino rabbits were randomly divided into four groups. Group I remained untreated and served as control. Group II was given gentamicin, group III was given gentamicin along with Salicylic acid (SA) and group IV was given only salicylic acid. The degree of hepatoprotection was measured by assessment of body weight, liver weight, absolute liver weight and estimations of plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), total and direct bilirubin, tissue malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) activities. Significant reduction in the elevated liver weight, plasma levels of AST, ALT, bilirubin & tissue MDA and significant elevation in reduced body weight, SOD and CAT activities were found that confirms the protective role of salicylic acid in gentamicin induced hepatotoxicity.

Keywords: Gentamicin sulphate, salicylic acid, liver biomarkers, antioxidant enzymes.

INTRODUCTION

Gentamicin (GM) is commonly applied in human clinical practices for treatment of life-threatening gram-negative infections (Graham *et al.,* 1997, Martin *et al.,* 2012). On the other hand, the efficacy of GM is restricted by the development of hepatotoxicity. In some cases, this side effect is so severe that the use of the drug must be discontinued. In spite of the introduction of newer and less toxic antibiotics, GM is still used clinically because of its quick bactericidal action, broad-spectrum activity, chemical stability, and low cost (Jakobsen *et al.,* 2007). GM-induced hepatotoxicity is characterized by hepatic cell necrosis, without morphological changes in hepatocytes structures (Pandit *et al.,* 2012). The mechanisms involved in GM-induced cell damage are not clearly understood. However, numerous studies demonstrated that reactive oxygen species (ROS) may be important mediators in GM-induced hepatotoxicity. Abnormal production of ROS directly damages some macromolecules and induces cellular damage and necrosis via numerous mechanisms including peroxidation of membrane lipids, protein denaturation and DNA damage (Kerksick *et al.,* 2005). Therefore the administration of numerous compounds with antioxidant activity has been successfully used to prevent or ameliorate GM-induced hepatotoxicity (Valko *et al.,* 2007).

In the past few years, much interest has been laid on the role of naturally occurring dietary substances for the control and management of diverse chronic diseases, one such compound salicylic acid (SA) has been used since ancient times to provide pain relief and treat inflammatory conditions. Salicylic acid is a phenolic compound present in plants, where it plays a central role in the development of local and systemic resistance to pathogen infection (Randjelovic *et al.,* 2011). Humans and animals obtain SA mainly from daily foods, fruits, and vegetables. Increasing evidence demonstrates that applied SA can neutralize oxidative damage induced by adverse conditions in animals, though the mechanisms underlying these effects remain unclear. It has been reported that SA comprises free radical-scavenging and iron chelation properties. SA can affect the activation of transcription factors, in particular nuclear factor kappa B (NF-κB), thereby intervening in apoptotic pathways. It is also a hydroxyl radical scavenger in both experimental animals and humans (Raskin 1992).

The aim of the present study was therefore to investigate the protective role of salicylic acid in gentamicin induced hepatotoxicity in rabbits.

MATERIALS AND METHODS

Animals

Twenty four adult male albino rabbits weighing about 1000gm to 2000gm were selected for study. These rabbits were purchased from a local market (Karachi, Pakistan) and housed in a temperature controlled (23±4°C) environment for 15 days before starting study, where rabbits were fed standard diet of alfalfa and water ad-libitum.

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Experimental protocol

Twenty four rabbits were divided into four experimental groups, six rabbits in each group and were placed into individual metabolic cages for study.

Group I: Control
Group II: Gentamicin sulphate (GS)
Group III: Gentamicin sulphate + salicylic acid (GS+SA)
Group IV: Salicylic acid (SA)

Animals of the group I remain untreated healthy animals and served as control. The group II was given gentamicin sulphate (80mg/kg i.p.) for 20 days. The group III was given gentamicin sulphate (80mg/kg i.p.) for 20 days along with Salicylic acid (SA) at the same dose, and the group IV was given only SA at the same dose. On day 21 the rabbits were sacrificed after weighing.

Plasma collection

The whole blood was collected in heparinized tubes. Thereafter plasma was separated by centrifugation at 2000rpm for 10mins and was stored at -70°C for analysis.

Body weight and organ weight

Body weight of each rabbit in a specific group was recorded before and after the experimentation. On the basis of initial and final weight, change in body weight was calculated. After dissection from the ventral side liver was removed, rinsed in cold saline, weighted and relative liver weight was determined for each rabbit.

Biochemical analysis

Assessment of plasma ALT, total and direct bilirubin

Plasma ALT (Retiman and Franhel, 1957), total and direct bilirubin (Sherlock, 1951) and ALP were analyzed using commercially prepared reagent kits from Randox.

Preparation of post mitochondrial supernatant

Liver homogenate was prepared by taking 1g of liver tissue in 10ml of 5mM potassium phosphate buffer (pH 7.8) 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 SOD, Catalase and MDA.

Estimation of thiobarbituric acid substances

The malondialdehyde (MDA) content, a measure of lipid peroxidation, was assayed in the form of thiobarbituric acid reacting substances (TBARS) by the lipid peroxidation method (Okhawa *et al.,* 1979). Briefly, the reaction mixture consisted of 0.2ml of 8.1% sodium dodecyl sulphate, 1.5ml of 20% acetic acid solution adjusted to pH 3.5 with sodium hydroxide and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid was added to 0.2ml of 10%(w/v) of PMS. 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 and pyridine (15:1 v/v) was added and centrifuged. The organic layer was taken out and its absorbance was measured at 532 nm and compared with those obtained from MDA standards. The concentration values were calculated from absorption measurements as standard absorption.

Estimation of catalase

Catalase activity was assayed by the method of Sinha (Sinha *et al.,* 1979). Briefly, the assay mixture was consisted of 1.96ml phosphate buffer (0.01M, pH 7.0), 1.0 ml hydrogen peroxide (0.2M) and 0.04 ml PMS (10%w/v) in a final volume of 3.0ml. 2ml dichromate acetic acid reagent was added in 1ml of reaction mixture, boiled for 10 minutes, cooled. Changes in absorbance were recorded at 570 nm.

Estimation of SOD

Superoxide dismutase levels in the cell free supernatant were measured by the method of (Kono *et al.,* 1978). Briefly 1.3ml of solution A (0.1m EDTA containing 50 mM Na_2CO_3, pH 10.0), 0.5ml of solution B (90µmNBTnitro blue tetrazolium dye) and 0.1ml of solution C (0.6% Triton X-100 in solution A), 0.1ml of solution D (20mM Hydroxylamine hydrochloride, pH 6.0) were mixed and the rate of NBT reduction was recorded for one minute at 560nm. 0.1ml of the supernatant was added to the test cuvette as well as reference cuvette, which do not contain solution D. Finally, the percentage inhibition in the rate of reduction of NBT was recorded as described above. One enzyme unit was expressed as the inverse of the amount of protein (mg) required in one minute.

Ethical approval

Ethical guidelines of institutional ERB (Ethical Review Board) were considered in conductance of experimental work, laboratory use and in animal care. The present research proposal is approved by the ethical review committee (ERC No.2004).

STATISTICAL ANALYSIS

The values were presented as means ± SD of different groups. Differences between the mean values were estimated using one way ANOVA SPSS version 22. The results were considered statistically significant when *$p<0.01$ and **$p<0.05$.

RESULTS

Effect of gentamicin sulphate and salicylic acid induction on liver weight and relative liver weight in experimental rabbits

Increase in liver weight and relative liver weight were observed in GS treated group as compare to control (GS: 35.125±1.109 $p<0.05$) (GS: 0.028±0.001 $p<0.05$).while rabbits of GS+SA group showed significant reduction in

Table 1: Effect of gentamicin sulphate and salicylic acid induction on body weight, absolute liver weight and relative liver weight in experimental rabbits

Groups	Body Weight (gm)	Absolute Liver Weight (gm)	Relative Body Weight (% liver to body weight)
Control	1358.429±34.733	32.875±1.974	0.023±0.002
GS	1262.619± 33.479*	35.125±1.109**	0.028±0.001**
GS+SA	1265.524±19.423*	30.750±2.901**	0.025±0.003**
SA	1313.952±15.002*	29.500±1.826**	0.022±0.003**

liver weight and relative liver weight (GS+SA: 30.750 ±2.901 p<0.05) (GS+SA: 0.025±0.003 p<0.05) as compare to group of GS treated rabbits. The group of rabbits treated with SA only showed reduction in liver weight and relative liver weight as compare to control (GS+SA: 29.500±1.826 p<0.01)(GS+SA: 0.022 ±0.003 p<0.05) as shown in table 1.

Table 2: Effect of gentamicin sulphate and salicylic acid induction on plasma ALP in experimental rabbits.

Groups	ALP(U/L)
Control	90.75±11.16
GS	95.75±3.49*
GS+SA	71.5±10.23*
SA	90.5±5.31

n=6 values are mean ± SD. Significant difference among control, gentamicin sulphate treated, gentamicin and salicylic acid treated and salicylic acid treated groups by one way ANOVA *p<0.01 and **p <0.05.

Table 3: Effects of Gentamicin sulphate and salicylic acid induction on plasma ALT in experimental rabbits.

Groups	ALT (U/L))
Control	40.75±5.58
GS	57.75±7.49**
GS+SA	31.25±11.51**
SA	40.25±13.86

Table 4: Effect of gentamicin sulphate and salicylic acid induction on direct bilirubin level in experimental rabbits.

Groups	Direct Bilirubin (mg/dl)
Control	0.25±0.05
GS	0.27±0.04**
GS+SA	0.15±0.05**
SA	0.25±0.05

Table 5: Effect of gentamicin sulphate and salicylic acid induction on plasma total bilirubin in experimental rabbits.

Groups	Total Bilirubin (mg/dl)
Control	0.3±0.15
GS	0.5±0.07**
GS+SA	0.25±0.11*
SA	0.3±0.01

Table 6: Effect of gentamicin sulphate and salicylic acid induction on hepatic concentrations of malondialdehyde in experimental rabbits.

Groups	MDA (nmol/gm)
Control	4406.663±374.2627
GS	5394.995±218.4617**
GS+SA	4144.997±572.3472**
SA	4025.498±611.2849**

Table 7: Effect of gentamicin sulphate and salicylic acid induction on hepatic concentration of superoxide dismutase in experimental rabbits.

Groups	SOD (unit/g)
Control	10.17±1.22
GS	4.65±0.45*
GS+SA	8.95±0.53*
SA	11.55±3.73*

Table 8: Effect of gentamicin sulphate and salicylic acid induction on catalase activity in experimental rabbits

Groups	Catalase (nmol/gm)
Control	1244.25±54.28
GS	1152.5±12.99*
GS + SA	1129.75±12.04*
SA	1244±53.26

Effect of gentamicin sulphate and salicylic acid induction on plasma ALP in experimental rabbits

Concentration of ALP was markedly increased in the GS treated group as compared to control (95.75±3.49, P<0.05). Salicylic acid administration in GS+SA group reduced the concentration of ALP as compare to GS treated group (71.5±10.23, P<0.01) while salicylic acid treated group showed a normal range of plasma ALP as compared to control (90.5±5.31) (table 2).

Effect of gentamicin sulphate and salicylic acid induction on plasma ALT in experimental rabbits

Plasma ALT was markedly increased in the GS-treated group as compared to control (57.75±7.49, P<0.05). Salicylic acid administration in GS+SA group significantly reduced plasma ALT as compare to GS treated group (31.25±11.51, P<0.05) while salicylic acid treated group showed normal range of ALT level as compare to control (40.25±13.86) (table 3).

Effect of gentamicin sulphate and salicylic acid induction on plasma direct bilirubin in experimental rabbits

Direct bilirubin was significantly increased in the GS-treated group as compared to control (0.275±0.043, P<0.05). The GS+SA group after Salicylic acid supplementation showed reduced direct bilirubin level (0.15±0.05, P<0.05) as compared to the GS treated group. Salicylic acid treated groups showed normal levels of direct bilirubin as compared to control (0.25±0.05) (table 4).

Effect of gentamicin sulphate and salicylic acid induction on plasma total bilirubin in experimental rabbits

Total bilirubin level was markedly increased in the GS-treated group as compared to control (0.5±0.07, P<0.05). Salicylic acid administration in GS+SA group reduced the concentration of total bilirubin as compare to GS treated group (0.25±0.11, P<0.01) while salicylic acid treated group showed almost normal total bilirubin level as compare to control (0.3±0.01) (table 5).

Effects of gentamicin sulphate and salicylic acid induction on hepatic concentration of MDA in experimental rabbits.

Level of tissue MDA was markedly increased in the GS-treated group as compared to control (5394.995±218.4617 P<0.05). Salicylic acid administration in the GS+SA group showed significantly reduced concentration of MDA as compared to the GS treated group (4144.997± 572.3472, P<0.05). The Salicylic acid treated group showed reduced MDA level as compared to the control group (4025.498±611.2849, P<0.05) (table 5).

Effect of gentamicin sulphate and salicylic acid induction on hepatic concentration of SOD in experimental rabbits

SOD activity was significantly reduced in the GS treated group as compared to control (4.65±0.45, P<0.01). GS+SA group, after salicylic acid supplementation, showed elevated levels of SOD activity (8.95±0.53, P<0.01) as compared to the GS treated group. SOD activity was slightly increased in the salicylic acid treated group (11.55±3.73, P<0.01) as compared to control.

Effect of gentamicin sulphate and salicylic acid induction on hepatic concentration of catalase in experimental rabbits

Concentration of catalase was markedly decreased in the GS treated group (1152.5±12.99) as compared to control. Administration of salicylic acid in the GS+SA treated group increased catalase level (1129.75±12.04, P<0.01) as compared to control. Salicylic acid treated showed normal catalase value as compared to control (1244±53.26) (table 8).

DISCUSSION

Hepatotoxicity or liver damage is the irregular functioning of the liver; with mitochondrial dysfunction reported as one of the major mechanisms of drugs that induce hepatotoxicity. By severely altering mitochondrial function in the liver, drugs can induce hepatic necrosis, causing cytolytic hepatitis, and can progress into liver failure (Jain *et al.,* 2010).

Body weight is an important indicator of adverse effects of antibiotics as well as it is considered as a determinant parameter of toxicity testing. The data indicates that the GS at the dose of 80mg/kg for 20 days decreases the body weight of the test (GS) group. Patel reported the treatment of GS at the dose of 80mg/kg i.p in male albino rats reduces the body weight as compared to control (Patel *et al.,* 2017). However Considerable weight gain observed in the GS+SA group indicates that SA attenuates the effect of gentamicin on body weight of rabbits.

Administration of gentamicin significantly (p<0.05) decreased the body weight, while increase the absolute and relative weights of liver in GS treated group as compared to control group this is also evident by Umbreen Rashid who reported that treatment of GM (80 mg/kg i.p) increased the absolute and relative liver weight of GM treated group as compared to control group. It has been proposed that gentamicin induced toxicity might be produced by oxidative damage in the liver and thus increase the weight of the organ. However, treatment with salicylic acid reduces the toxicity of gentamicin as well as reverting the liver weight towards the control values suggesting the protective role of salicylic acid being an antioxidant (Rashid *et al.,* 2017). AST predominantly found in mitochondria of hepatocytes. ALT is more specific to the liver, and thus is a better parameter for detecting liver injury. Serum bilirubin is also associated with liver cell damage. The ALT, AST and serum bilirubin levels are largely used as most common biochemical markers to evaluate liver injury (Greish *et al.,* 2009). The significant decrease in activities of these enzymes by salicylic acid may indicate the anti-necrotic effect on the liver. This may be due to the fact that the antioxidants offer protection and maintain the functional integrity of hepatic cells. Bilirubin is released from the destroyed red blood cells and passed on to the liver. The liver excretes the bilirubin in the fluid called bile. If the liver is not functioning properly, the bilirubin will not be properly excreted. Therefore, if the bilirubin level is higher than normal, it may mean that the liver is not functioning correctly (Baranano *et al.,* 2002). The amount of albumin in gentamicin treated animals showed a significant decrease compared to the control group (Mehmood *et al.,* 2014). Serum bilirubin is considered an index for the assessment of hepatic function and any abnormal increase indicates hepatobiliary disease and severe disturbance of

hepatocellular architecture (Martin *et al.,* 1992). Gentamicin administration resulted in increased serum bilirubin level, thereby suggesting severe hepatic injury and confirming the hepatotoxic nature of gentamicin. Treatment with salicylic acid significantly decreased the elevated level of total bilirubin in serum towards normalcy indicating its hepatoprotective efficacy. Treatment with salicylic acid recovered the injured liver to normal which indicates that salicylic acid being an antioxidant has anti-hepatotoxic effect. Gentamicin enhanced the production of superoxide anion, hydrogen peroxide and hydroxyl radicals by mitochondria (Yang *et al.,* 1995). Free radicals cause Peroxidation of phospholipid membranes, DNA strand breakage, and protein denaturation. Treatment of rats with salicylic acid effectively decreased liver enzyme levels in the blood. This can be attributed to the presence of phenolic compounds in salicylic acid that can act by scavenging free radicals (Abdel-Wahhab *et al.,* 2005). 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 stended to normalize the level of MDA. A role of lipid peroxidation in gentamicin-induced nephrotoxicity has also been described in previous studies. Injection of gentamicin at a toxic dose induced a marked hepatic failure, characterized by significant increase in lipid peroxidation. Gentamicin injection at a dose of (80mg/kg b.wt.) induced liver 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 toxicity (Kumar *et al.,* 2000). The hepatoprotective effect of salicylic acid may be due to salicylic acid reducing lipid-peroxidation by direct antioxidant effect.

In the current study, GM induced oxidative stress which results in decrease in antioxidant enzymes like catalase and superoxide dismutase (SOD). There is some experimental data suggesting that toxic drugs may also change levels of MDA, CAT & SOD (Ozbek *et al.,* 2000) which are commonly used to monitor the development and extent of cellular damage due to oxidative stress. Thus, the preventive effect of salicylic acid induces decrease in the activity of super oxide dismutase (SOD) and CAT that could be attributed to the restoration of markers of liver injury. It seems reasonable to assume that salicylic acid is able to suppress toxicity in the liver.

CONCLUSION

Protective role of salicylic acid in gentamicin induced hepatotoxicity was confirmed through significant reduction in increased liver weight, levels of plasma bilirubin, ALT, AST and tissue MDA and a marked elevation in decreased body weight, SOD and catalase activities.

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REFERENCES

Abdel-Wahhab MA and Aly SE (2005). Antioxidant property of *Nigella sativa* (black cumin) and *Syzygium aromaticum* (clove) in rats during aflatoxicosis. *J. Appl. Toxicol.,* **25**(3): 218-223.

Aboubakr M and Abdelazim AM (2016). Hepatoprotective effect of aqueous extract cardamom against gentamicin induced hepatic damage in rats. *Int. J. Basic Appl. Sci.,* **5**(1): 1.

Appel G. B. (1990). Aminoglycoside nephrotoxicity. Am J Med, 88(3), S16-S20.

Baranano DE, Rao M, Ferris CD and Snyder SH (2002). From the Cover: Biliverdin reductase: A major physiologic cytoprotectant. *PNAS,* **99**(25): 16093-16098.

El-Ashmawy IM, El-Nahas AF and Salama OM (2006). Grape seed extract prevents gentamicin induced nephrotoxicity and genotoxicity in bone marrow cells of mice. *Basic Clin. Pharmacol. Toxicol.,* **99**(3): 230-236.

Girish C, Koner BC, Jayanthi S, Ramachandra Rao K, Rajesh B and Pradhan SC (2009). Hepatoprotective activity of picroliv, curcumin and ellagic acid compared to silymarin on paracetamol induced liver toxicity in mice. *Fundam. Clin. Pharmacol.,* **23**(6): 735-745.

Jain A and Singhai AK (2010). Effect of *Momordica dioica* roxb on gentamicin model of acute renal failure. *Nat Prod Res,* **24**(15): 1379-1389.

Kono Y (1978) Generation of Superoxide Radical during Autoxidation of Hydroxylamine and an Assay for Superoxide Dismutase. *Arch Biochem. Biophys.,* **186**: 189-195.

Kumar KV, Naidu MUR, Shifow AA and Ratnakar KS (2000): Probucol protects against gentamicin-induced nephrotoxicity in rats. *Indian J. of Pharmacol.,* **32**(2): 108-113.

Mahmood, N, Haleh M, Mohammad P, Mohsen F and Hossein KJ (2015). Pathological changes of gentamicin in liver tissue and antioxidant properties of Cinnamon extract on Wistar rats. *BPJ,* **7**(1): 341-347.

Martin P and Friedman LS (2018). Assessment of liver function and diagnostic studies. *In*: Handbook of Liver Disease. Elsevier, pp. 1-17.

Mayuren C, Reddy VV, Priya, SV and Devi VA (2010). Protective effect of Livactine against CCl4 and paracetamol induced hepatotoxicity in adult Wistar rats. *N. Am. J. Med. Sci.,* **2**(10): 491.

Müller N, Schiller P and Ackenheil M (1991). Coincidence of schizophrenia and hyperbilirubinemia. *Pharmacopsychiatry,* **24**(06): 225-228.

Ohkawa H, Ohishi N and Yagi K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem.,* **95**(2): 351-358.

Ozbek E, Turkoz Y, Sahna E, Ozugurlu F, Mizrak B, Ozbek M (2000). Melatonin administration prevents the nephrotoxicity induced by gentamicin. *BJU Int.,* **85**: 742-746.

Patel R and Shah JG (2017). Protective effect of ethanolic extract of Hordeum vulgare seed on gentamicin induced nephrotoxicity. *Int. Res. J. Pharm.,* **8**(8): 1-6.

Randjelovic P, Veljkovic S, Stojiljkovic N, Jankovic-Velickovic L, Sokolovic D, Stoiljkovic M and Ilic I (2012). Salicylic acid attenuates gentamicin-induced nephrotoxicity in rats. *Sci. World J.,* 390613.

Rashid U and Khan MR (2017). *Fagonia olivieri* prevented hepatorenal injuries induced with gentamicin in rats. *Biomed. Pharmacother.,* **88**: 469-479.

Reitman S and Frankel S (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.,* **28**(1): 56-63.

Sinha A (1972). Colorimetric Assay of Catalase. *Anal. Biochem.,* **47**: 389-394.

Yang and Han (1995). Renal cortical mitochondria are the source of oxygen free radicals enhanced by gentamicin. *J. Clin. Invest,* **8**: 334-341.