Original Article

Nephroprotective Effect of Metformin on Gentamicin Induced Renal Injuries in Rats

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ABSTRACT

Objective: Metformin, an oral anti-diabetic agent has been studied in the past for its protective effects as an antioxidant. The study was conducted to compare the nephroprotective effects of two doses of metformin in aminoglycoside induced renal injuries in Sprague-Dawley rats.

Study Design: experimental study

Place and Duration of Study: This study was conducted at the Department of Pharmacology, ANMCH, Islamabad, Pakistan in collaboration with the animal house of National Institute of Health (NIH),Islamabad, Pakistan from July 2015 to December 2015.

Materials and Methods: 30 adult male Sprague-Dawley rats weighing 200 ± 50 g, kept under similar conditions for food and temperature, were randomly divided into three groups of Gentamicin treated control (C), Gentamicin & low dose Metformin treated 1 (M1) and Gentamicin & high dose Metformin treated 2 (M2). Renal failure was induced by injecting gentamicin (80mg/kg/day) intraperitoneally for eight days followed by oral metformin for 28 days. Sampling for serum creatinine, 24 hour urinary volume and 24 hour urinary proteins was done at day 0, 14 and 28.

Results: It was found that metformin administration in gentamicin induced renal failure results in significantly increased urinary output, reduced creatinine levels along with significantly decreased urinary proteins in M2 group as compared to M1 group. While in group C, all parameters of renal failure were increased.

Conclusion: Metformin in a dose of 150mg/kg prevents aminoglycoside-induced renal injuries in rats.

Key Words: Metformin, Gentamicin, Nephrotoxicity, Renal injuries, Nephroprotective effect

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INTRODUCTION

Kidneys play a vital role to maintain homeostasis. Renal injuries secondary to medications are not uncommon resulting in varying degree of damage from minor injury to complete renal failure. The extent of renal damage depends upon the dose and the duration of exposure to these drugs or substances[1, 2].

Gentamicin is an aminoglycoside derived from actinomycete Micromonospora .Therapeutically it is used for uncomplicated infections of lower urinary tract. It is often combined with β-lactam antibiotics for better penetration in the cells to treat hospital acquired pneumonias and multi drug resistant infections[3].

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In order to develop acute renal failure in animals for research purposes ,several animal models have been developed by administering higher doses of drugs such as glycerol (single dose10mg/kg IM) [4], gentamicin (80 mg/kg per day IP) for 7 days [5],non-steroidal anti-inflammatory drugs (NSAIDs) (single dose 3g/Kg PO) [6,7].

Aminoglycosides' nephrotoxicity (including gentamicin & others) usually manifest as acute renal failure with some abnormalities on urinalysis[8].The glomerular filtration rate is reduced and proteins and hyaline and granular casts start appearing in the urine. Serum creatinine levels start rising. Inhibition of enzymes which further bring changes in the functions of mitochondria and ribosomes, leading to decrease formation of membrane derived prostaglandins, inositol phosphate and diacylglycerols is postulated mechanism of nephrotoxicity with gentamicin[9].

Metformin is usually used as a first line drug in type-2 diabetes mellitus. Metformin increases the insulin sensitivity and reduces glucose production by the liver as a result the circulating insulin levels are reduced[10]. Metformin reduces the oxidative stress and cell death by a mechanism which is dependent on a special type of pore opening in mitochondria. This pore is known as mitochondrial permeability transition pore

(PTP).Metformin also reduces the production of reactive oxygen species (ROS) in endothelial cells by inhibition of protein kinase C. Metformin inhibits hepatic glucose formation by increasing the small heterodimer partner (SHP) gene expression with the help of adenosine monophosphate-activated protein kinase (AMPK)-dependent pathway[11].The rationale of this study was to investigate protective effect of metformin as an antioxidant with two different doses i.e., 75 mg/kg and 150mg/kg, given by gavage tube to the rats in whom renal failure was induced by gentamicin.

MATERIALS AND METHODS

This experimental study was conducted at Department of Pharmacology, ANMCH, Islamabad, Pakistan in collaboration with animal house of National Institute of Health (NIH), Islamabad, Pakistan from July 2015 to December 2015. Healthy male Albino rats (Sprague-Dawley) weighing 200 ± 50 grams, were purchased from National Institute of Health Islamabad and kept at animal house of the same institute. Animals were kept in cages of 2x3 feet size at standard conditions. Animals (N=30) were divided into 03 groups of 10 rats in each group.

Control Group (Group C) was given gentamicin 80mg/kg[5] intraperitoneally from 0- 8 days along with distilled water (01 ml) by gavage from 0- 28 days as single morning dose.

Experimental Group I (Group M1) was given gentamicin 80mg/kg intraperitoneally from 0-8 days along with metformin (75mg/kg/day) dissolved in 01ml distilled water by gavage from 0-28 days as single morning dose.

Experimental Group II (Group M2) was given gentamicin 80mg/kg intraperitoneally from 0-8 days along with metformin (150mg/kg/day)[12] dissolved in 01ml distilled water by gavage from 0- 28 days as single morning dose.

Rats were placed in individual metabolic cages 24 hour prior to collection of urine. 24 hour urine samples were collected in glass flasks on day 0, 14 and 28 of the study .Urine volume was measured with the help of disposable syringes .24 hour urinary proteins and serum creatinine were measured using reagent kit. 1 ml of blood was taken by direct cardiac puncture on day 0, 14 and 28 after anesthetizing the animal with Chloroform. Data was analyzed by using SPSS (Version 19.0). Mean±SD values were calculated for each group. To calculate the descriptive statistics, one way ANOVA (Tukey's test) was applied. P-value ≤ 0.05 was considered as significant.

RESULTS

Mean ± SD values of serum creatinine of Sprague-Dawley rats at day 0, 14 and 28 of the experiment are shown in table 1.

Table No.1: Mean ± SD of values of Serum Creatinine (mg/dL) of Sprague-Dawley rats at day 0, 14 and 28 of experiment. (N=30)

Groups	Dose of drugs	Serum Creatinine (mg/dl)		
		Sampling Day 0	Sampling Day 14	Sampling Day 28
Control group (c)	Gentamicin (80mg/kg)	0.68 ± 0.20	1.82 ± 0.33	3.46 ± 1.35
Treated group 1 (m1)	Gentamicin (80mg/kg) + metformin (75mg/kg)	0.64 ± 0.15	1.72 ± 0.49	1.50 ± 0.41
Treated group 2 (m2)	Gentamicin (80mg/kg) + metformin (150mg/kg)	0.64 ± 0.08	1.78 ± 0.33	0.98 ± 0.19

Results of M2 group indicate that serum creatinine value was increased at 14th day as compared to 0 day value of this group but it was insignificantly lower than the 14th day value of the control group. Also when compared with the same day value of the M1 group, the difference again was statistically insignificant ($p>0.05$). The increasing trend was reversed at day 28 of the experiment where the value was found to be 0.98 ± 0.19 mg/dL showing a highly significant decrease ($p<0.005$) in comparison to the same day value of control group and reversing the serum creatinine levels very close to the normal values at day 0. When serum creatinine value of M2 group was compared with that of M1 group at day 28 of the experiment, they showed statistically significant difference ($p<0.05$) confirming that metformin in double dose (150 mg/Kg body weight) has more pronounced nephroprotective effect.

Table No.2: Mean ± SD of values of 24 hour urinary volume (mL) of Sprague-Dawley rats at day 0, 14 and 28 of experiment. (N=30)

Groups	Dose of Drugs	24 Hour Urinary Volume (ml)		
		Sampling Day 0	Sampling Day 14	Sampling Day 28
Control group (c)	Gentamicin (80mg/kg)	3.43 ± 0.68	1.69 ± 0.24	1.10 ± 0.40
Treated group 1 (m1)	Gentamicin (80mg/kg)+ metformin (75mg/kg)	3.93 ± 0.61	2.57 ± 0.76*	2.01 ± 1.10
Treated group 2 (m2)	Gentamicin (80mg/kg)+ metformin (150mg/kg)	3.65 ± 0.84	2.24 ± 0.47	3.27 ± 0.61

Table-2 shows that in M2 group the urinary volume was decreased at day 14 as compared to day 0 value of this group but it was significantly higher than the 14th day value of control group. The value increased at day 28 to 3.27±0.61 mL showing a highly significant increase in urine output in comparison to the same day value of control group. The value at day 28 in M2 group was also close to day 0 values.

Table No.3: Mean ± SD of values of 24 hour proteins (mg/dL) of Sprague-Dawley rats at day 0, 14 and 28 of experiment. (N=30)

Groups	Dose of Drugs	24 Hour Urinary Proteins (mg/dl)		
		Sampling Day 0	Sampling Day 14	Sampling Day 28
Control group (c)	Gentamicin (80mg/kg)	11.84 ± 2.24	60.06 ± 15.48	68.50 ± 15.16
Treated group 1 (m1)	Gentamicin (80mg/kg)+ metformin (75mg/kg)	10.25 ± 1.74	35.23 ± 5.28**	30.60 ± 11.18
Treated group 2 (m2)	Gentamicin (80mg/kg)+ metformin (150mg/kg)	11.28 ± 2.40	19.79 ± 4.02**†	15.05 ± 3.33

Table-3 shows that at day 28 of the experiment, the 24 hour urinary proteins value was found to be 15.05±3.33 mg/dL showing a highly significant decrease ($p<0.005$) in comparison to the same day value of control group and reversing the 24 hour urinary proteins level very close to the normal values at day 0. When 24 hour urinary protein value of M2 group was compared with that of M1 group at day 28 of the experiment, they showed statistically significant difference ($p< 0.05$) confirming that metformin in a dose of 150mg/Kg body weight has more pronounced nephroprotective effect in gentamicin induced renal injuries.

DISCUSSION

Many clinically used drugs adversely effects renal system.[1,2] Nephrotoxicity with Gentamicin results in acute renal failure and manifests as abnormalities of urinalysis.[8] The powerful antioxidant effect of Metformin acts by diminishing oxidative stress and inhibit the aminoglycoside mediated acute renal failure by a mitochondrial dependent pathway .It can also prevent gentamicin induced renal injuries in rats[13].

In previous studies, efforts have been done to prevent renal damage by introducing Metform orally mixed in drinking water making it difficult to identify the exact dose of drug the animal has consumed as the gentamicin induced toxicity causes the rats to become inactive or anorexic[5]. So this experiment was designed to study the effects of two different doses of metformin i.e., 75 mg/kg and 150mg/kg, given by gavage tube to the rats in whom renal failure was induced by gentamicin.

Udupa and his colleague evaluated the effect of gentamicin in rats using urinary biomarkers and noticed significant change in serum BUN, creatinine level and urinary proteins which are also observed in our study. They used two doses of gentamicin 30 and 100mg/Kg/day where as we administered 80mg/Kg dose of gentamicin [14].

The rate of reduction in urinary volume with gentamicin toxicity in our study was similar to the findings of Morales et al .Their study proved that metformin prevent gentamicin induced renal toxicity by mitochondrial dependent pathway which restores its functions and also normalize oxidative stress[15] .In our study, urinary volume showed increasing trend in both metformin treated groups, especially in M2 group, at day 28 of experiment, 24 hour urinary volume of rats was close to the base line levels seen at day 0 .This proves the renoprotective effect of Metformin in our study which is also explained by Frid with his colleagues. They mentioned in their study that metformin increases the total antioxidant status (TAS) along with the normalization of lipid peroxidation and prevent the renal damage caused by oxidative stress[16]. The reduction in 24 hour urinary proteins, serum creatinine induced by gentamicin with the larger doses of metformin in our study is supported by literature.[17]. Metformin also reduces the production of reactive oxygen species (ROS) in endothelial cells by modulation of p38 mitogen–activated protein kinase expression which further contributes to its renoprotective effect.[18]

CONCLUSION

Based on the results of our study it was concluded that metformin at a dose of 150mg/kg produced a nephroprotective effect in gentamicin induced renal injuries in Sprague-Dawley rats.

Author's Contribution:

Concept & Design of Study:	Sohail Ahmed
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Conflict of Interest: The study has no conflict of interest to declare by any author.

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