Protective effect of metformin against gentamicin induced nephrotoxicity in rabbits

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Abstract: Gentamicin is used against gram negative infections, but major problem encountered is nephrotoxicity that occurs in 10-20% of therapeutic regimes. Gentamicin induced oxidative stress plays an important role in this nephrotoxicity. Recent data has shown metformin to possess antioxidant activity. Purpose of study was to evaluate potential role of metformin in protecting kidney from nephrotoxicant insult. Thirty-six rabbits were randomly divided into six groups (n=6). G-1 received 1 ml isotonic saline intraperitoneally (IP) daily for 13 days. G-2 received gentamicin (150 mg/kg/day) IP for last 6 days of 13 days. G-3 received gentamicin (40 mg/kg/day) IP for 13 days. G-4 received metformin salt (100 mg/kg/day) dissolved in drinking water via feeding tube for 13 days. G-5 received metformin salt (100 mg/kg/day) via feeding tube for 13 days plus gentamicin (150mg/kg/day) IP for last 6 days of 13 days. G-6 received gentamicin (40mg/kg/day) IP plus metformin salt (100mg/kg/day) via feeding tube for 13 days. Blood was collected on days 0 and 14 for serum urea & creatinine estimation. All animals were sacrificed and kidneys were removed for renal histological examination. Metformin showed nephroprotective effect. It blunted nephrotoxic insult at 150mg/kg/day of gentamicin, whereas showed complete nephroprotection at 40mg/kg/day of gentamicin. Metformin offers complete nephroprotection at low toxic dose ranges of gentamicin. This could offer an efficacious and cheaper treatment alternative in those diabetics who also suffer from gram-negative infections.

Keywords: Gentamicin, Metformin, Nephrotoxicity, Nephroprotection.

INTRODUCTION

Gentamicin, the prototype drug of aminoglycosides, is widely used against infections by gram-negative bacillary microorganisms (Mingeot-leclercq *et al.*, 1999), but the major clinical problem encountered during its use is nephrotoxicity that occurs in 10-20% of the therapeutic regimes (Quiros *et al*., 2011). Oxidative stress induced by gentamicin plays an important role in this nephrotoxicity (Koyner *et al*., 2008).

Gentamicin causes augmented synthesis of reactive oxygen and nitrogen species (Balakumar *et al*., 2010) by renal mitochondria (Kumar *et al.,* 2001) that affect the renal structural and functional integrity in due course of time (Sundin *et al*., 2001; Cuzzocrea *et al.,* 2002).

Gentamicin induced functional deterioration is reflected as escalation in serum levels of urea and creatinine beyond normal limits; loss of albumin and carnitine in urine; and decline in glomerular filtration rate (Romero *et al*., 2009). Structural changes include desquamation of epithelial cells; atrophic and hypertrophic changes in glomeruli; glomerular congestion; perivascular edema and edema of proximal tubules; and fibrotic and necrotic changes in tubules (Polat *et al*. 2006).

Metformin, a biguanide, is an oral antihyperglycemic agent extensively used for treating type 2 diabetes. It exerts pleiotropic actions on many tissues in the body (Palomba *et al*., 2009). Metformin exerts its principal metabolic action in the liver (Diamanti-Kandarakis *et al*., 2010) by regulation of glucose and fat metabolism. The probable mechanisms include diminished substrate uptake by liver for gluconeogenesis, inhibition of enzymes involved in gluconeogenesis and enhanced insulin receptor and insulin receptor substrate phosphorylation (Yuan *et al*., 2003; Ashokkumar and Pari, 2005; Gunton *et al*., 2003; Cheng *et al*., 2006; Mithieux *et al*., 2002; Lutz *et al*., 2001). It also facilitates hepatic glucose uptake and stimulates glycolytic kinases (glucokinase and pyruvate kinase) (Fulgencio *et al*., 2001). Metformin also decreases lipogenesis and increases fatty acid oxidation through inhibition of lipogenic enzymes, predominantly the activity of acetyl-CoA carboxylase (ACC).

In skeletal muscle, metformin enhances insulin signaling and activates AMP activated protein kinase (AMPK). The net result is reduced postprandial glucose levels and diminished systemic insulin resistance (Diamanti-Kandarakis *et al*., 2010).

In adipose tissue, metformin exerts antiadipogenic effect. It inhibits lipogenesis and stimulates AMPK-dependent fatty acid oxidation (Diamanti-Kandarakis *et al*., 2010).

In the ovary metformin prevents overproduction of sex steroids and premature luteinization by blocking effects of excessive insulin on follicular growth and

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steroidogenesis, thus also improving ovulation. In addition, metformin-induced AMPK activation also enhances antioxidant defenses at the ovarian tissue level (Diamanti-Kandarakis *et al*., 2010).

Metformin also has beneficial effects on endothelium. By AMPK activation, metformin treatment suppresses the production of proinflammatory cytokines. In addition it also enhances production of endothelial nitric oxide synthase (eNOS) and nitric oxide (NO) in endothelial cells. This helps in deceleration of atherosclerotic injury (Diamanti-Kandarakis *et al*., 2010).

Lately recent research has unveiled several newer domains of metfromin's action that produce potential beneficial effects in stressful conditions.

Metformin improves mitochondrial homeostasis, thereby diminishes apoptosis induced by oxidative stress and prevents cell death. It boosts the antioxidant system by complementing mitochondrial function. It significantly decreases the depletion of respiratory components (cytochrome c, NADH) (Morales *et al*., 2010).

METHODS

The study was conducted in the department of Pharmacology, Army Medical College, Rawalpindi.

Animals

Thirty six healthy male and non-pregnant female domestic rabbits were used for the study. Animals were 7 – 10 months of age and weighed between 1 and 1.9kg at the beginning of study. They were kept in the animal house of Army Medical College, Rawalpindi for a week for acclimatization before starting the experiment. Temperature of animal house was maintained at 24-26°C. Diet of the rabbits consisted of grass, grain, seasonal vegetables and water *adlibitum*. Twelve hours light & dark cycle was maintained.

Drugs

1. Injection Gentamicin (containing 80 mg of gentamicin sulfate in 2 ml solution)
2. Pure salt of Metformin

Commercially available gentamicin formulation for clinical applications was used in present study (Beauchamp *et al*., 1997; Beauchamp *et al*., 1991). Gentamicin injections (2ml solution containing 80mg of gentamicin sulfate) from Reckitt & Coleman Pharmaceuticals, Karachi, Pakistan were acquired from local pharmacy. Like other pharmaceutical preparations of gentamicin, it contained a mixture of three main components, gentamicin C1, C1a and C2. Werrick Pharmaceuticals, Islamabad, Pakistan donated the pure salt of metformin.

Experimental design

Thirty-six rabbits were randomly divided into six experimental groups, each group containing six rabbits. Gentamicin was given parenterally by intraperitoneal route (Weir *et al.,* 1990). Metformin was given by mouth mixed in drinking water via feeding tube. The drugs were given for 13 days following the underlying regime.

G-1 (Control group) was given isotonic saline in dose of 1 ml intraperitoneally once everyday (Beauchamp *et al*, 1997) for 13 days.

G-2 was housed for 13 days and administered gentamicin injection in nephrotoxic dose of 150mg/kg/day intraperitoneally in last 6 days of the 13 day period (Morales *et al*., 2010).

G-3 was given gentamicin injection intraperitoneally in nephrotoxic dose of 40 mg/kg/day (Yasin *et al.,* 2003) for 13 days.

G-4 received the antidiabetic drug metformin (100 mg/kg/day) dissolved in drinking water via feeding tube for 13 days (Morales *et al*., 2010).

G-5 received the antidiabetic drug metformin (100 mg/kg/day) dissolved in drinking water via feeding tube for 13 days plus gentamicin injection (150 mg/kg/day) intraperitoneally for last 6 days of 13 days (Morales *et al*., 2010).

Group-6 received injection of gentamicin (40 mg/kg/day) (Yasin *et al.,* 2003) intraperitoneally plus metformin salt (100mg/kg/day) dissolved in drinking water via feeding tube for 13 days (Morales *et al*., 2010).

Collection of samples

Blood samples of rabbits were collected twice during the study i.e. at the start on day 0 and then at the end on day 14. Body weights were also recorded on day 0 & day 14. Blood sample was drawn from the tip of the ear from the marginal ear vein. Dorsal surface of the ear was shaved and disinfected with 70% alcohol. Xylene was then applied to dilate the vessel. Gentle stroking was also done occasionally for vasodilation. When the vessel became prominent, blood was withdrawn with the help of butterfly needle of 24 G size. About 2ml of blood was taken and then stored in a centrifuge tube. Sterilized cotton swab was then pressed firmly on the vessel to stop bleeding. Alcohol was then applied followed by washing with soap and water to remove xylene. The blood sample was permitted to clot at room temperature. It was then centrifuged for 15 minutes at 3000 rpm. An automatic micropipette was used to separate the serum at -20^{o}C in fresh dry serum storage vial for urea and creatinine estimation. The instrument used was Automatic chemistry analyser selectra E.

Twenty-four hours after the administration of last doses of drugs, on day14, rabbits were sacrificed by giving a cut with a sharp blade on the ventral side of the neck severing the carotid arteries. Then immediately after death, the abdomen was slit open with help of a longitudinal

incision to take out both kidneys. Renal capsules were removed carefully and kidneys were sagitally divided. The slices were then placed in 10% formalin for 24 hours. This was followed by renal tissue processing for paraffin embedding. A rotary microtome was used to make 3-5µm thick sections, which were then mounted on glass slides. Hematoxylin and Eosin (H&E) stains were used for tissue staining and sections were histologically examined under light microscope paying more emphasis to proximal tubules (Ozbek *et al*., 2010).

Biochemical analysis

Biochemical analysis was carried out by automated enzymatic assays. Urea was estimated by Urease/kinetic method (Burtis and Ashwood, 1999) and serum creatinine by Jaffe reaction (Young, 2001).

Histopathology

Rabbits were sacrificed twenty-four hours after giving the last dose of the drug (Ozbek *et al*., 2010). Kidneys were taken out, sections were made and further processed for histopathologic examination (Hewitt *et al*., 2008; Anderson and Bancroft, 2002*).*

Grading Criteria for Tubular Injury on Microscopic Examination

Histological sections of kidneys from all rabbits were qualitatively and quantitatively assessed. Microscopic examination was carried out. Specimens were examined for tubular necrosis, which was graded as follows (Houghton *et al.,* 1978; Derakhshanfar *et al*., 2009):

0-No necrosis; 1- Mild necrosis involving single cell in thinly scattered tubules; 2-Moderate, necrosis involving more than one cell in sparse tubules; 3-Marked necrosis with tubules showing complete necrosis in almost every single power field; and 4- Massive complete necrosis.

STATISTICAL ANALYSIS

Results were shown as means ±S.E.M. ONE WAY ANOVA was applied to determine statistically significant differences, followed by Post Hoc Tukey test using SPSS software version 16. Percent weight change was calculated using Microsoft office Excel 2007. Statistical significance was indicated by $P<0.05$.

'Chi-Square Test' was used to analyze results of histopathology. $P<0.05$ was taken as statistically significant.

RESULTS

All the animals survived the experimental period of fourteen days.

Observation of individual parameters

Body weight

Mean body weight in G-1 (given saline) increased by 2.26 ±0.26 percent of the initial weight. In G-2 (nephrotoxic control given 150mg/kg/day of gentamicin), mean body weight fell by 16.5±0.9 percent. G-3 (nephrotoxic control given 40mg/kg/day of gentamicin) showed 10.41±1.20 percent weight loss from initial weights. In G-4 (metformin control given 100mg/kg/day of metformin), 2.09±0.22 percent weight loss from initial body weights was observed. G-5 (given 100mg/kg/day of metformin and 150mg/kg/day of gentamicin) showed 7.73±0.49 percent and G-6 given 100mg/kg/day of metformin and 40mg/kg/day of gentamicin) showed 1.3±0.42 percent weight loss (table 1).

Serum urea

Serum urea remained within the normal range over the 14 day experimental period in the animals of G-1 that received saline and served as control. It was 6.38±0.53 mmol/l on day 14 (table 2). This biochemical marker also stayed in normal range in the group that received metformin alone (G-4). The value was 6.71±0.11 mmol/l (table 2). P values were found statistically insignificant when G-4 was compared with G-1 ($P>0.05$) (table 5).

In comparison to G-1, serum urea increased markedly i.e. upto 25.45±1.84 mmol/l in G-2 that received 150 mg/kg/day of gentamicin (table 2). P value was significant when compared with G-1 (25.45±1.84 of G-2 versus 6.38±0.53 of G-1; $P<0.05$) (table 3).

G-3 received 40 mg/kg/day of gentamicin and serum urea levels went up to 12.71±1.31 mmol/l (table 2). P value was <0.05 when compared with control group (table 4).

The nephroprotective effects as well as extent of protection of antidiabetic drugs metformin against gentamicin induced renal insult was assessed in groups 5 and 6. The animals of G-5 were treated with metformin 100mg/kg/day and gentamicin 150mg/kg/day and the serum urea value was 13.68±2.11 mmol/l on day 14 (table 2). When compared with G-2 i.e. the nephrotoxic group that received 150mg/kg/day of gentamicin (25.45± 1.84 mmol/l), metformin mitigated the rise of serum urea in G-5 and the difference between G-2 and G-5 was statistically significant ($P<0.05$) (table 6).

G-6 received metformin 100mg/kg/day and gentamicin 40 mg/kg/day and the serum urea value was 6.96±0.09 mmol/l (table 2). When compared with G-3 i.e. the nephrotoxic group that received 40 mg/kg/day of gentamicin (12.71±1.31 mmol/l), the difference was statistically significant ($P<0.05$) (table 7).

Serum creatinine

Like serum urea, serum creatinine also remained within normal limits in G-1and G-4 over the 14 day experimental period (table 2 and 5).

Serum creatinine increased significantly at the end of study period in high nephrotoxic dose gentamicin group

Table 1: Mean change in body weight of different groups of rabbits over 14 days study period

Group	Mean Initial Weight (kg)	Mean Final Weight (kg)	Mean Weight Gain (%)	Mean Weight Loss (%)	± SEM
Group 1 (treated with normal saline)	1.32	1.35	2.26	-	0.26
Group 2 (treated with gentamicin 150 mg/kg/day)	1.57	1.31	-	16.50	0.99
Group 3 (treated with gentamicin 40mg/kg/day)	1.28	1.14	-	10.41	1.20
Group 4 (treated with metformin 100 mg/kg/day)	1.42	1.39	-	2.09	0.22
Group 5 (treated with metformin 100 mg/kg/day+ gentamicin 150 mg/kg/day)	1.49	1.38	-	7.73	0.49
Group 6 (treated with metformin 100 mg/kg/day+ gentamicin 40 mg/kg/day)	1.53	1.51	-	1.30	0.42

Table 2: Serum parameters of different groups of rabbits on day 0 and day 14

Groups	Serum Urea (mmol/l)				Serum Creatinine (µmol/l)			
	Day 0	± SEM	Day 14	± SEM	Day 0	± SEM	Day 14	± SEM
Group 1 (treated with normal saline)	6.96	0.16	6.38	0.53	104.33	2.48	104	3.61
Group 2 (treated with gentamicin 150 mg/kg/day)	6.28	0.37	25.45	1.84	94.83	3.56	373.83	2.42
Group 3 (treated with gentamicin 40mg/kg/day)	6.38	0.48	12.71	1.31	107	4.15	140.33	5.70
Group 4 (treated with metformin 100 mg/kg/day)	6.6	0.23	6.71	0.11	104.5	2.36	105	2.29
Group 5 (treated with metformin 100 mg/kg/day+ gentamicin 150 mg/kg/day)	7.23	0.15	13.68	2.11	113.16	5.53	208.16	5.91
Group 6 (treated with metformin 100 mg/kg/day+ gentamicin 40 mg/kg/day)	6.88	0.13	6.96	0.09	99.16	3.93	102.16	4.51

Table 3: Effect of 150 mg/kg /day of gentamicin on renal functions of rabbits (n=6)

DAYS	DAY-0		DAY-14	
TEST	G-1	G-2	G-1	G-2
Serum urea (mmol/l)	6.96	6.28	6.38	25.45
SEM	±0.16	±0.37	±0.53	±1.84
P value <0.001*				
Serum creatinine(µmol/l)	104.33	94.83	104	373.83
SEM	±2.48	±3.56	±3.61	±2.42
P value <0.001*				

Table 4: Effect of 40 mg/kg /day of gentamicin on renal functions of rabbits (n=6)

DAYS	DAY-0		DAY-14	
TEST	G-1	G-3	G-1	G-3
Serum urea (mmol/l)	6.96	6.38	6.38	12.71
SEM	±0.16	±0.48	±0.53	±1.31
P value 0.002*				
Serum creatinine (µmol/l)	104.33	107	104	140.33
SEM	±2.48	±4.15	±3.61	±5.70
P value < 0.001*				

P value <0.05 = Significant (*)
P value >0.05 = Not significant (Ns)

Table 5: Effect of 100 mg/kg/day of metformin on renal functions of rabbits (n=6)

DAYS	DAY-0		DAY-14	
TEST	G-1	G-4	G-1	G-4
Serum urea (mmol/l)	6.96	6.6	6.38	6.71
SEM	±0.16	±0.23	±0.53	±0.11
P value 0.999^{Ns}				
Serum creatinine (µmol/l)	104.33	104.5	104	105
SEM	±2.48	±2.36	±3.61	±2.29
P value 1.000^{Ns}				

Table 6: Comparison between renal effects of 150 mg/kg /day of gentamicin alone and combination of 100 mg/kg/day of metformin and 150 mg/kg /day of gentamicin (n=6)

DAYS	DAY-0		DAY-14	
TEST	G-2	G-5	G-2	G-5
Serum urea (mmol/l)	6.28	7.23	25.45	13.68
SEM	±0.37	±0.15	±1.84	±2.11
P value 0.001*				
Serum	94.83	113.16	373.83	208.16
SEM	±3.56	±5.53	±2.42	±5.91
P value < 0.001*				

Table 7: Comparison between renal effects of 40 mg/kg /day of gentamicin alone and combination of 100 mg/kg/day of metformin and 40 mg/kg /day of gentamicin (n=6)

DAYS	DAY-0		DAY-14	
TEST	G-3	G-6	G-3	G-6
Serum urea (mmol/l)	6.38	6.88	12.71	6.96
SEM	±0.48	±0.13	±1.31	±0.09
P value 0.001*				
Serum	107	99.16	140.33	102.
SEM	±4.15	±3.93	±5.70	±4.51
P value < 0.001*				

P value < 0.05 = Significant (*)
P value > 0.05 = Not significant (Ns)

Table 8: Comparison of histopathological findings of different groups P value < 0.001 by applying Chi-Square Test)

Groups	Grade-0 necrosis	Grade-1 necrosis	Grade-2 necrosis	Grade-3 necrosis	Grade-4 necrosis
1	+	---	---	---	---
2	---	---	---	+	---
3	---	---	+	---	---
4	+	---	---	---	---
5	---	---	+	---	---
6	+	---	---	---	---

i.e. G-2 (373.83±2.42 µmol/l) and low nephrotoxic dose group i.e. G-3 (140.33±5.70 µmol/l) (table 2) as compared to G-1. P value was also statistically significant (P<0.05) (tables 3 & 4). In G-5 that received 100mg/kg/day metformin and high nephrotoxic dose gentamicin, serum creatinine was 208.16±5.91µmol/l (table 2). On comparison with high nephrotoxic dose gentamicin group i.e. G-2 (373.83±2.42 µmol/l), P value was statistically significant (P<0.05) (table 6).

G-6 which received 100 mg/kg/day metformin and low nephrotoxic dose i.e. 40 mg/kg/day gentamicin, serum creatinine was 102.16±4.51 µmol/l (table 2). On comparison with low nephrotoxic dose gentamicin control group i.e. G-3 (140.33±5.70 µmol/l), difference was significant (102.16±4.51 µmol/l of G-6 versus 140.33± 5.70 µmol/l of G-3, P<0.05) (table 7).

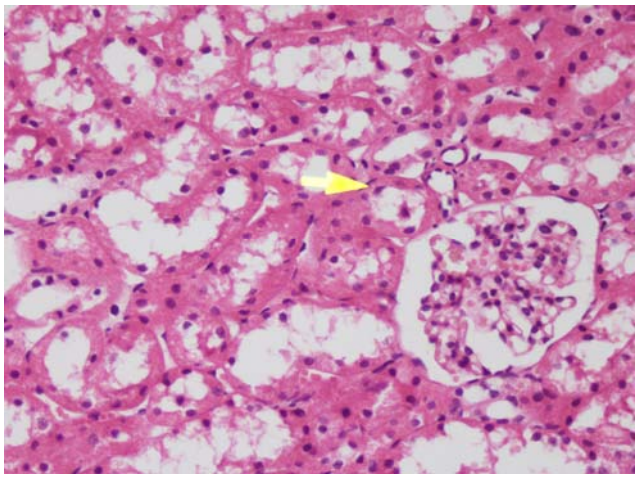

Fig. 1: Light micrographs showing normal histology of proximal renal tubules in G-1 saline treated rabbits (400X)

Histological examination

Histological examination of the renal sections of rabbits from the control group i.e. G-1 was normal (grade 0 necrosis). No cell necrosis was detected in any power field (fig. 1).

Microscopic examination of G-2 (treated with 150 mg/kg/day of gentamicin) revealed marked grade 3 necrosis (fig. 2) in 83% of the rabbits of this group. Massive necrosis and cellular desquamation was found in more than 50% of the proximal renal tubules in almost every power field in these renal sections. However, intact tubules were also identified. Interstitium was found to be infiltrated with diverse inflammatory cells together with a number of eosinophils exhibiting hypersensitivity reaction type III, predominantly in the areas of necrotic tubules. Moderate grade 2 necrosis was observed in the rest of 17% of the animals of the group characterized by tubular epithelial necrosis and desquamation in only sparse tubules.

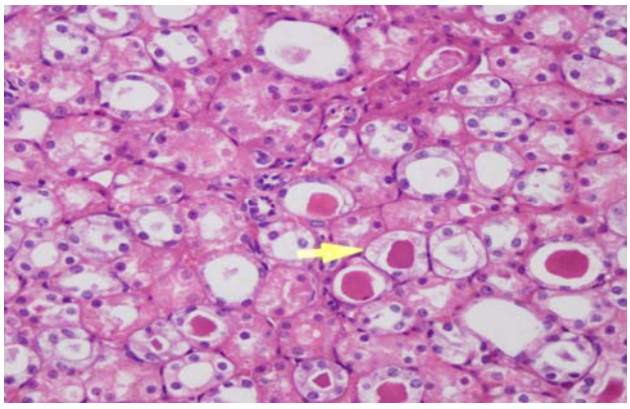

Fig. 2: Marked necrosis in proximal tubules with hyaline cast and loss of nuclei observed in rabbits of G-2 treated with 150 mg/kg/day gentamicin (400X)

67% of the rabbits in G-3 (given 40 mg/kg/day of gentamicin) exhibited moderate grade 2 necrosis (fig. 3). The remainder 33% animals showed mild grade 1 necrosis marked by single cell necrosis in rare proximal tubules. Areas of focal epithelial cell degeneration with or without evidence of desquamation were seen in these animal's renal sections.

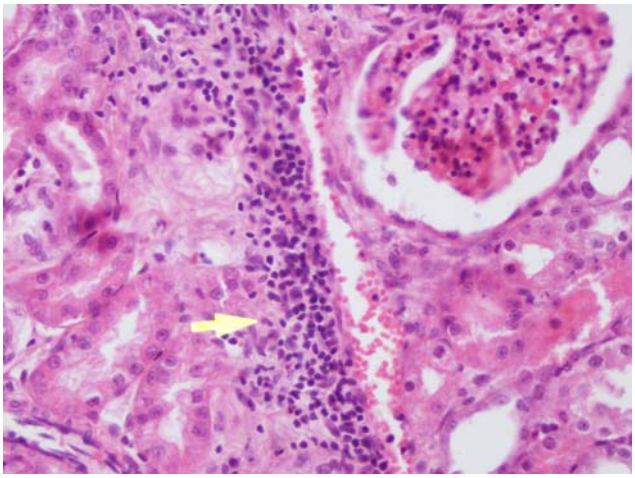

Fig. 3: 40mg/kg/day gentamicin treated G-3 showing inflammatory cells infiltration, congestion and tubular necrosis in renal tubules (400X)

Histological examination of G-4 (given 100 mg/kg/day of metformin alone) showed normal finding without any evidence of necrosis (fig. 4).

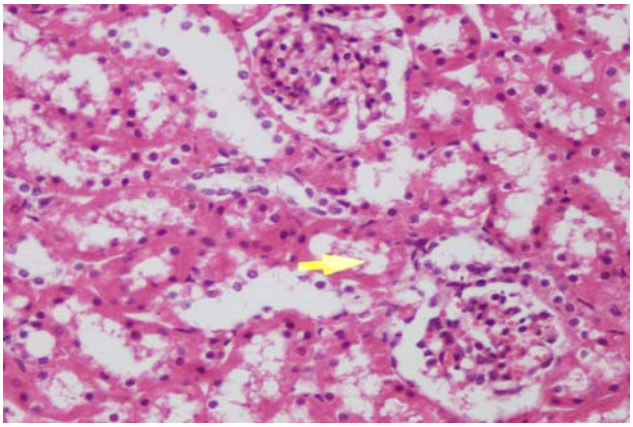

Fig. 4: Normal morphology of renal tubules in metformin alone treated animals of G-4 (400X)

In G-5 (treated with 100mg/kg/day of metformin and 150 mg/kg/day of gentamicin), moderate grade 2 necrosis (fig. 5) was observed in 66%, mild to moderate necrosis in 16 % and mild necrosis in 17% of the animals. Microscopic examination of the sections of kidneys from G-6 (treated with 100mg/kg/day of metformin and 40 mg/kg/day of gentamicin) was found to be normal with absence of signs of necrosis (fig. 6).

When we applied the chi square test, 'P' value was <0.001, which showed the significant difference among the groups (table 8).

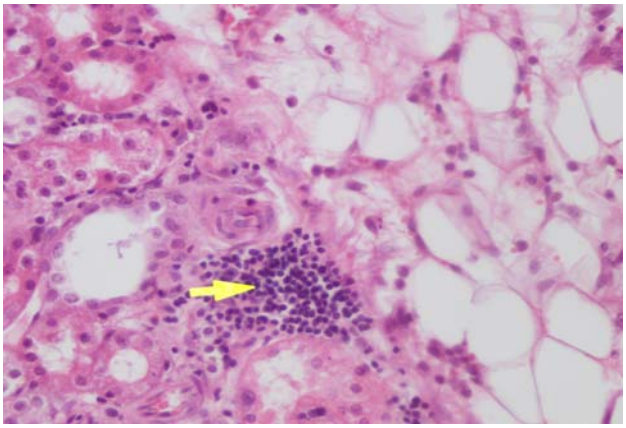

Fig. 5: Interstitial inflammation and tubular epithelial necrosis observed in sparse renal tubules in animals of G-5 treated with metformin and gentamicin 150mg/kg/day (400X)

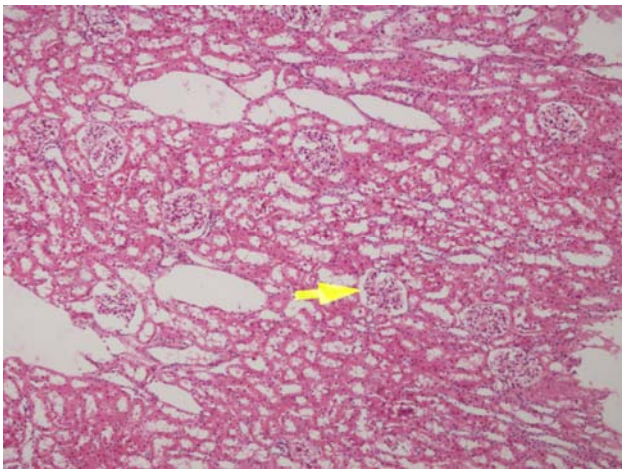

Fig. 6: Normal histology in sections of rabbits from G-6 treated with metformin and gentamicin 40mg/kg/day revealing almost complete prevention of gentamicin induced histopathological alterations by biguanide (X100)

DISCUSSION

The present research project has been carried out to study the nephrotoxicant insult induced by two diverse doses i.e.150mg/kg/day and 40mg/kg/day of gentamicin, the prototypical and the most widely used drug among aminoglycoside group of antibiotics, and to determine the renoprotective potential of antidiabetic drug metformin when it was used concurrently with gentamicin. The study also aimed to determine the extent of nephroprotection provided by the metformin at these two nephrotoxic doses.

In our study the group of animals that received 150 mg/kg/day nephrotoxic dose of gentamicin showed highly deranged serum urea (P□0.001) and serum creatinine (P□0.001) values in comparison to the control group. The histological exam of the kidneys also exhibited grade 3 necrosis predominantly. Mean body weight of the group animals fell too by 16 percent. Comparable changes were observed by many other researchers following use of 150mg/kg/day nephrotoxic dose of gentamicin (Morales *et al.,* 2010). Gossrau (1989) also reported analogous alterations in the tubular apparatus after administering 150 mg/kg/day of the gentamicin to the rats. Erdem and the coworkers (2000) observed the similar weight loss in experimental animals following administration of nephrotoxic dose of gentamicin. Body weight loss resulted from decreased oral intake that occurred following acidosis and increased catabolism associated with gentamicin induced acute renal failure (Ali *et al.,* 1992).

The other nephrotoxic group that received 40mg/kg/day of gentamicin also showed moderately abnormal serum urea (P□0.002) and serum creatinine (P□0.001) values. The renal histology revealed mainly grade 2 necrosis. The animals of the group showed 10 percent weight loss from initial weights. These findings are consistent with the results of Chaware *et al.,* (2011) and Yasin *et al.,* (2003), who also used 40mg/kg/day of the gentamicin to induce nephrotoxicity in rats.

In our study, metformin has shown variable levels of nephroprotection. The group that received high i.e.150mg/kg/day nephrotoxic dose of gentamicin with 100mg/kg/day of metformin did not show complete nephroprotection as indicated by the serum urea and serum creatinine values which were out of the normal ranges. However comparison with the nephrotoxic control group that received 150mg/kg/day of gentamicin showed attenuation of the nephrotoxicant insult, as evidenced by comparative decline in values of serum urea (P□0.001) and serum creatinine (P□0.001). Likewise the animals of this group also showed weight loss of about 7.6 percent which when compared with the nephrotoxic control group that received 150mg/kg/day of gentamicin was less (7.6 percent versus 16.5 percent).

On the other hand, the group that received 40mg/kg/day of gentamicin with 100mg/kg/day of metformin exhibited complete nephroprotection as serum urea and serum creatinine of these animals remained within normal ranges. This was further complemented by the histological examination of the kidneys, which didn't show any alteration in the renal morphology. Change in weight of the animals was also only about 1 percent. Furthermore all these impressive effects were observed at a dose of metformin that corresponds to the clinically demonstrated therapeutic range (Wilcock and Bailey, 1994). The slight weight loss observed in this group can be attributed to decreased net caloric intake due to the

appetite suppressing effect of metformin treatment (Yki-Ja¨rvinen *et al.,* 1999).

Metformin has gained latest popularity due to its newly discovered effects on cellular and mitochondrial functions (Guigas *et al.,* 2004; Zou *et al.,* 2004). The latest research has shown this antidiabetic agent to moderately reduce oxidative stress induced apoptosis in the endothelial cell (EI-Mir *et al.,* 2008; Morales *et al.,* 2010). Nephroprotective effect of metformin has also been observed by many researchers. Alhaider and the co-researchers (2011) reported attenuation of diabetic nephropathy in rats through modulation of oxidative stress by metformin. Giugas (2004) showed inhibition of cell death and apoptosis by metformin in a pharmacological in-vitro study. Morales (2010) confirmed the similar antioxidant and antiapoptotic effect in *in vitro* study using renal cells. The study by Beeson (2010) showed metformin to restore the cell vitality in the cultured renal cells when exposed to nephrotoxicants gentamicin, cisplatin and mercuric chloride. The results of the present study lend further credence to the nephroprotective effect of metformin.

The current study, therefore, concludes that metformin offers complete nephroprotection at moderately toxic doses of gentamicin but it only ameliorates the nephrotoxicant insult at very high toxic doses of gentamicin. This, however, is in contrast to the findings of Morales (2010). He and the co-workers suggested that metformin could offer complete nephroprotection against gentamicin induced nephrotoxicity. This inconsistency could be due to the difference in the species of the study animals as Morales used rats for his experiments whereas the rabbits were used in the current study.

Over the past years a lot of work has been done to explore the pleiotropic effects of metformin (Diamanti-Kandarakis *et al.,* 2010). Its role in the prevention and management of diabetic nephropathy has been explored and confirmed by many researchers (Alhaider *et al.,* 2011; Xu *et al.,* 2011). But no study has been done to explore its nephroprotective effects at different doses of gentamicin.

It can be inferred from present study that metformin can offer complete nephroprotection at low toxic dose ranges of gentamicin. This could prove quite useful in diabetic patients who also suffer from gram-negative infections, as they are already vulnerable to kidney damage. So metformin could be an efficacious and cheaper treatment alternative to these patients, while preventing the renal toxicity and maintain the blood sugar as well.

At very high toxic doses of gentamicin, protection from renal damage is there but it is not complete. This is probably explained by the fact that some other mechanisms of aminoglycoside-induced nephrotoxicity may come into play at very high toxic doses of gentamicin, which couldn't be suppressed by metformin.

CONCLUSIONS

Gentamicin, a highly efficient antibiotic against gram-negative infections, is a potentially nephrotoxic drug and the severity of nephrotoxic insult escalates with increasing doses of the drug.

Metformin, an antidiabetic drug of the Biguanide class, has a preventive role against gentamicin induced renal dysfunction. Concomitant administration of this drug with gentamicin prevents the antibiotic induced biochemical and histological kidney injuries. The degree or extent of this protective effect varies with dose of gentamicin. At very high toxic doses of gentamicin, metformin may only blunt the nephrotoxic insult. However at low toxic doses of gentamicin, metformin offers complete nephroprotection. Furthermore this protective effect is observed at a dose of metformin that corresponds with the clinically demonstrated therapeutic range.

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