Original Article

Histological Effects of Glutamine on Gentamicin-Induced Nephrotoxicity in Wistar Rats

Ayesha Khalid, Saira Aslam, Ammara Ghafoor, Sania Asif

ABSTRACT

Objective: To determine the protective effects of glutamine on gentamicin-induced nephrotoxicity.

Methodology: This was a randomized control study which was conducted at the University of Health Sciences, Lahore. Total 36 rats, aged 6-8 weeks with body weights between 170-200 g were divided into four equal groups A to D by random balloting method. Group A was the control group and was given distilled water only by oral gavage for six days. Group B was given gentamicin for six days intraperitoneally. Group C was given a single dose of glutamine by oral gavage and were sacrificed after 24 hours. Group D was given both glutamine and gentamicin intraperitoneally for six days. The animals of groups A, B & D were sacrificed at the end of the experiment on the 7th day and then kidneys were excised from rats and processed for histological examination.

Results: Histological parameters of groups A, C, and D showed that proximal convoluted tubules (PCT) and distal convoluted tubules (DCT) of the kidney appeared normal. There were no signs of necrosis in epithelial lining, broken basement membrane as well as disrupted brush borders. In group B, gentamicin administration resulted in the deformation of glomeruli with thickened basement membrane and widening of urinary space. Also, degenerative changes were seen in PCT in the gentamicin group.

Conclusion: The nephrotoxicity and the oxidative damage caused by gentamicin on the kidneys of rats were incredibly significant. The use of glutamine supplements had shown promising results in providing protective effects on kidneys in the presence of gentamicin in rats.

Keywords: *Glutamine. Gentamicin. Rats.*

INTRODUCTION

Kidney function is crucial for the maintenance of homeostasis in the human body. Being a vital organ, it has an important role in detoxification and regulation of the acid-base balance. In addition, kidneys also play role in the synthesis and regulation of erythropoietin essential for blood pressure regulation.[1]

Gentamicin is an effective aminoglycoside antibiotic used for the treatment of a variety of bacterial infections. Its long-term use in clinical practice is limited due to renal damage and oxidative stress. The onset of renal toxicity is usually at a lower rate that is evident by a slow rise in daily serum creatinine levels as compared to the renal failure caused by other agents. Renal function markers usually increase after 7 to 10 days of the start of treatment with aminoglycosides and in more than half of the patients renal damage occurs after the completion of the dose.[2]

Nephrotoxic changes induced by gentamicin in the proximal convoluted tubules (PCT) result from its lysosomal internalization which eventually causes cell necrosis and tubular obstruction by the release of hydrolase enzymes.[3] Gentamicin affects the kidneys as it is retained in the epithelial linings of proximal convoluted tubules of the kidneys after glomerular filtration. This accumulation of gentamicin in the epithelial cells damages the kidneys and hinders their normal functioning, which include the removal of wastes from the blood, regulating the balance of electrolytes in the body, and maintaining the levels of fluids in the body. In the kidneys, like other aminoglycosides, gentamicin also produces reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and hydroxyl radical of renal cortical mitochondria which directly attack and damage the linings of the kidneys and cause an increase in lipid peroxidation.[4]

A variety of antioxidants have been used to minimize the oxidative damage caused by gentamicin and provide a protective effect on the kidneys. Glutamine, a non-essential amino acid, is considered a promising antioxidant and a useful compound to cope with the nephrotoxic effects of gentamicin. Glutamine acts as an immuno-nutrient as it preserves the immune competence of the body by promoting wound healing after major surgical procedures.[5] Glutamine repairs the intestinal mucosal injury in ischemia-reperfusion by reducing the expression of high mobility group box 1 (HMGB1) and inflammatory cytokines and reducing the permeability of the intestinal mucosa.[6] A study on rats has shown that glutamine promotes antioxidant protection and reduces inflammation.[7]

The role of glutamine as a potential antioxidant and a useful ameliorating agent in minimizing gentamicin-induced nephrotoxicity has also been investigated. The administration of glutamine injections to rats who have undergone tubular necrosis, formation of renal casts, and exfoliation of tubular epithelial cells due to

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ischemic shock, leads to the expression of heat shock protein which relieves the symptoms of kidney damage.[8]

This study was designed to observe the gentamicin-induced nephrotoxic changes in proximal convoluted tubules and the effect of glutamine against gentamicin-induced nephrotoxicity in Wistar rats.

METHODOLOGY

A randomized control study was conducted at the University of Health Sciences, Lahore after the approval by the University ethical committee (Letter No: UHS/Education/110-19/3465, 20-12-2019) on 36 healthy, adult male Wistar rats of the age of 6-8 weeks. The study was conducted from January to March 2020. The animals were divided randomly, using the balloting method into four equal groups: A, B, C, and D containing 9 animals each. Rats were kept under standard conditions (temperature of 23±2 ºC, humidity of 55±5%) and were fed on a commercial standard rat diet. All procedures were carried out in a clean and aseptic environment approved by the ethical committee of the University. The weight of all the rats was recorded at the beginning and at the end of the experimental period. They were allowed to acclimatize 3-4 days before starting the experiment.

Group A was the control group and was given distilled water orally for 6 days only. Group B was given intraperitoneally 100 mg/kg body weight of gentamicin for six days. Group C was given a single dose of glutamine (300 mg/kg body weight) by oral gavage and was sacrificed after 24 hours. Group D was given a single dose of glutamine (300 mg/kg dissolved in 1 ml of distilled water) as pretreatment on day 0 by oral gavage. After 24 hours, 100 mg/kg of gentamicin was given intraperitoneally, dissolved in 0.75 ml isotonic solution for 6 days. The animals of groups A, B & D were sacrificed on the 7th day of the experiment. The kidneys were excised from rats and processed for histological examination. For histological examination small pieces of the tissues, 3-5 mm cube, were excised and processed. The hematoxylin and eosin (H & E) and periodic acid-Schiff (PAS) staining techniques were used to analyze the kidney tissues for the presence of nephrotic damage in the four groups.

STATISTICAL ANALYSIS

The data was entered and analyzed using Statistical Package for the Social Sciences (SPSS) version 22. For qualitative variables like changes in the basement membrane, epithelial necrosis, and intraluminal protein casts, frequencies and percentages were calculated. Chi-square test was applied to find the association between groups and histological parameters.

RESULTS

The hematoxylin and eosin stained sections of groups A (Figure 1a, 2a), C (Figure 1b, 2b), and D (Figure 1c, 2c) showed that proximal convoluted tubules (PCT) and distal convoluted tubules (DCT) of kidneys were normal. There were no signs of necrosis in epithelial lining (0% in group A and C, 11% in group D) and broken basement membrane (0% in group A and C, 22% in group D) as well as disrupted brush borders (0% in group A and C, 22% in group D) (Table 1). In group B, gentamicin administration resulted in the deformation of glomeruli with thickened basement membrane and widening of urinary space. Degenerative changes were seen in PCT; there was marked necrosis (89%) with partial loss of brush border (89%), vacuolation, desquamating cells, and luminal casts (33%) in PCT (Table 1, Figure 1d). At places, there were strips of necrosed tubules with complete obliteration of lumen and increased eosinophilia. The basement membrane was also disrupted in some sections of group B. Interstitium showed marked inflammation mostly perivascular infiltration of macrophages and lymphocytes. Almost all the intraglomerular and extraglomerular vessels were congested and hyalinized with thickened walls (Figure 2d).

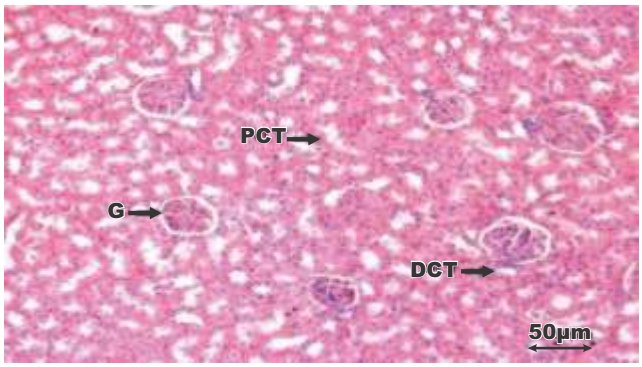

Figure 1a: Group A - Photomicrograph Showing PCT, Glomeruli (G), DCT, and Normal Interstitium (H & E stain, 100x magnification)

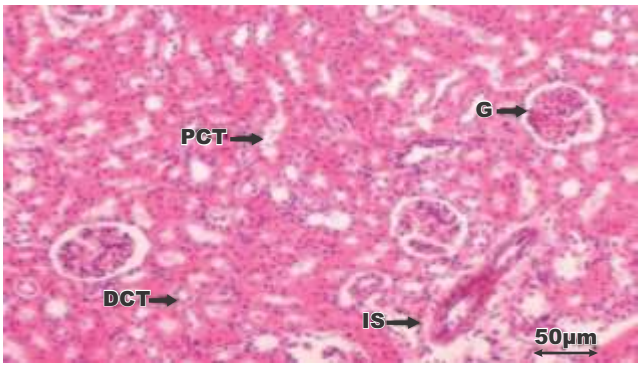

Figure 1b: Photomicrograph of Histological Section from Kidney of Group C Showing Normal Looking Glomeruli, PCT, DCT, and Normal Interstitium with a Blood Vessel (H & E stain, 100x magnification)

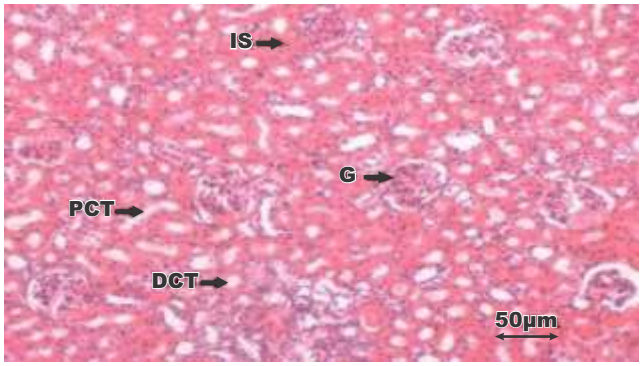

Figure 1c: Group D - Histological Section of Kidney Photomicrograph Showing Glomeruli (G), PCT, DCT, and Interstitium (IS). Slight Inflammation is Present (H & E stain, 100x magnification)

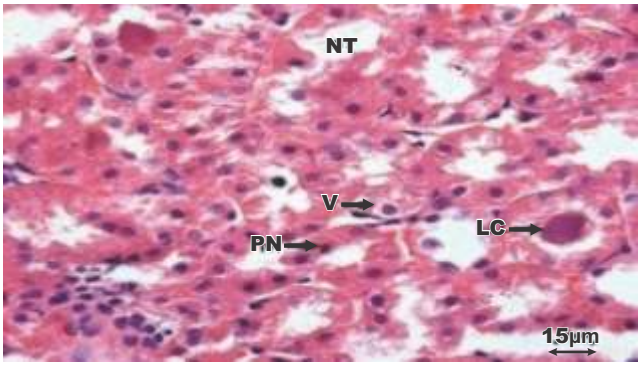

Figure 1d: Group B - Photomicrograph of Kidney's Cortex Showing Necrosed Tubules (NT) with Bigger Eosinophilia and Loss of Nuclei, Epithelial Cells with Vacuolar Degeneration (V) and Pyknotic Nuclei (PN). Tubular Lumen either Totally Obliterated or Filled with Casts (H & E stain, 100x magnification)

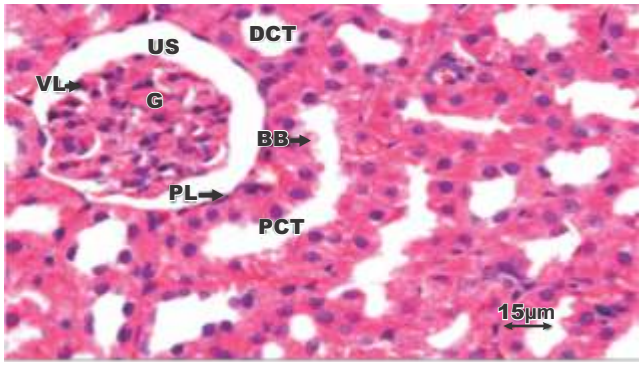

Figure 2a: Photomicrograph of the Cortical Part of Kidney of Group A Showing the Intact Brush Border (BB) of PCT, DCT, Parietal Layer (PL), Visceral Layer (VL), Urinary Space (US), and Glomerulus (G) (H & E stain, 400x magnification)

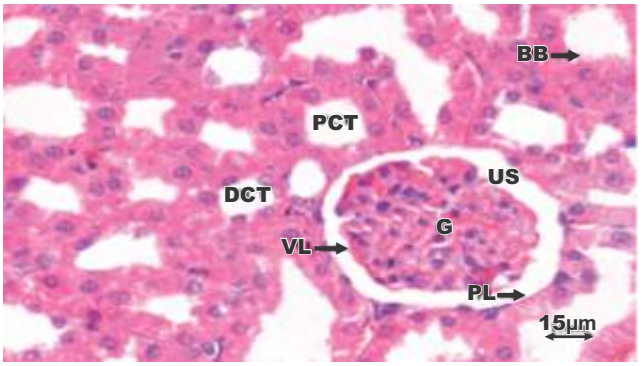

Figure 2b: Group C - Photomicrograph of Cortex of Kidney is Showing Normal Glomerulus (G) Enclosed by Visceral Layer (VL) & Parietal Layer (PL) of Bowman's Capsule having Urinary Space (US) in between. PCT with Prominent Brush Border (BB) (H & E stain, 400x magnification)

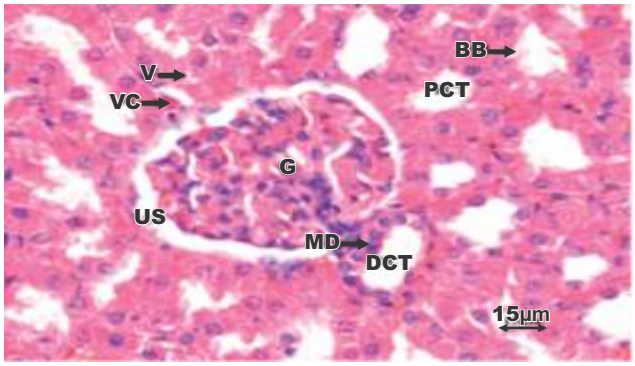

Figure 2c: Group D - Photomicrograph of Cortex of Kidney from Prophylactic Group Showing Glomerulus (G) Enclosed by Visceral and Parietal Layer of Bowman's Capsule having Urinary Space (US) in between. PCT Lined with Cuboidal Epithelium having Distinct Brush Border (BB), Vacuolations (V) Present in a Few Cells. DCT with Simple Cuboidal Epithelium Forming Macula Densa (MD) Near Vascular Pole of Renal Corpuscle. Slight Vascular Congestion (VC) is also Observable (H & E stain, 400x magnification)

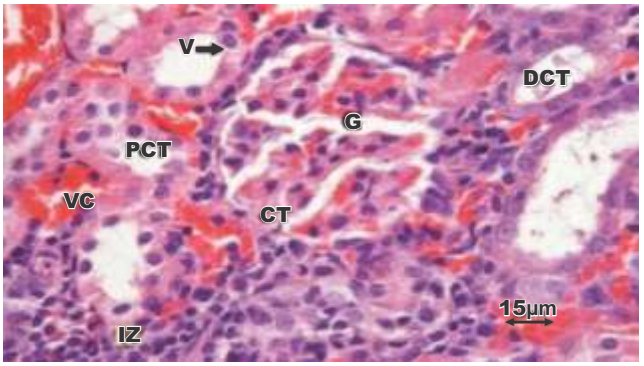

Figure 2d: Group B - Photomicrograph of Kidney's Cortex Showing Glomerulus (G), with Capillary Wall Thickening (CT) and Adhesions between Capillary Tuft and Bowman's Capsule. PCT Lined with Cuboidal Cells with Disrupted Cell Boundaries having Vacuolation (V) are Shown. DCT with Simple Cuboidal Epithelium. Interstitium Showing Inflammatory Zone (IZ) and Vascular Congestion (VC) (H & E stain, 400x magnification)

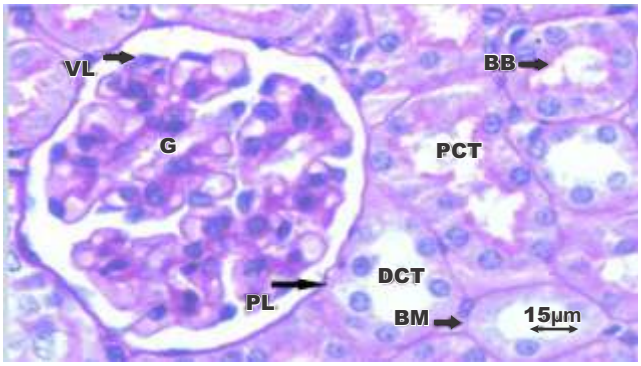

Figure 3a: Group A - Kidney Cortical Part is Shown in Photomicrograph. PCT are Intact with Brush Border (BB) Basement Membrane (BM) is Showing Normal Looking Parietal Layer (PL), Visceral Layer (VL), Urinary Space (US) and Glomerulus (G) (PAS stain, 400x magnification)

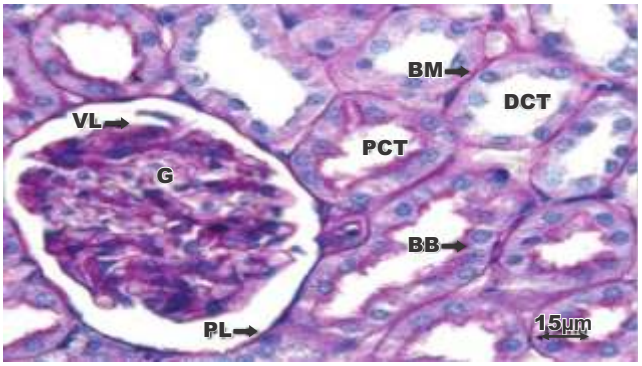

Figure 3b: Group C Shows a Photomicrograph of the Kidney's Cortex. We Observed Normal Glomerulus(G) Enclosed by Visceral (VL) and Parietal Layer (PL) of Bowman's Capsule having Urinary Space (US) between PCT with Prominent Brush Border (BB) and Basement Membrane of all the Tubules are Intact Including Glomerulus (PAS stain, 400x magnification)

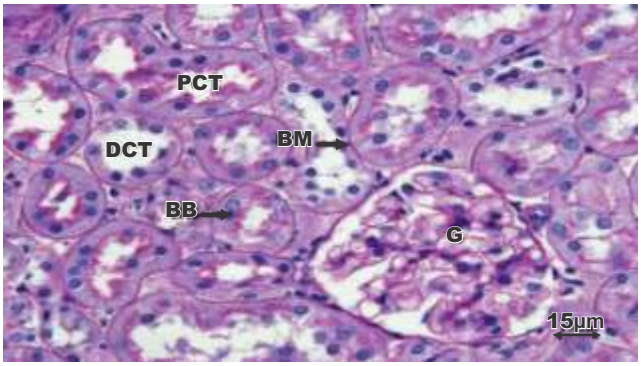

Figure 3c: Group D is Showing a Photomicrograph of the Kidney's Cortex. Normal Glomerulus (G) Enclosed by Bowman's Capsule. PCT are Lined with Simple Cuboidal Epithelium having Prominent Brush Border (BB) and Basal Membrane (BM) are Observed. The Presence of DCT with Simple Cuboidal Epithelium is Shown and Basement Membrane is Intact (PAS stain, 400x magnification)

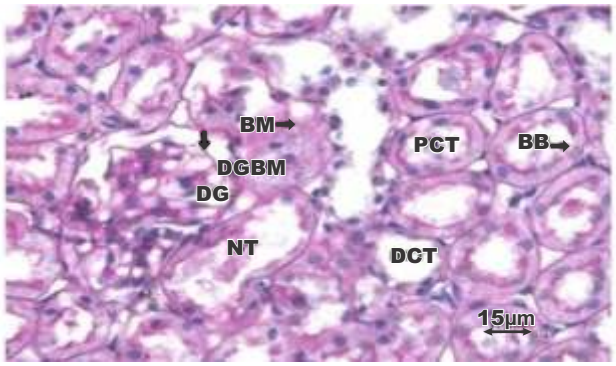

Figure 3d: Photomicrograph of Cortex of Kidney from Gentamicin Treated Group B. Deformed Glomerulus (DG), Disrupted Glomerular Basement Membrane (DGBM); PCT, Basal Membrane (BM) having Degenerating Cell Boundaries with Loss Of Brush Border (BB) having Vacuolation are Observed (PAS stain, 400x magnification)

The periodic acid-Schiff (PAS) stained sections of groups A (Figure 3a), C (Figure 3b), and D (Figure 3c) showed no sign of nephrotic damage. There were no pathologies seen in the kidney tissues and all the parts of the kidney were intact (Figure 3a). Analysis of PAS stained kidney tissues from group B animals showed indistinct PCT with epithelial necrosis, vacuolization, destruction of brush border membrane, presence of intraluminal protein casts, and damage to the basement membrane, and also the presence of vacuolization and inflammation as well as vascular congestion (VC) which were not observed in other three groups (Figure 3d).

Table 1: Comparison of Histopathological Parameters among Groups A, B, C, and D

Parameters	Group A		Group B		Group C		Group D		p-value
	Present	Absent	Present	Absent	Present	Absent	Present	Absent	
Disrupted Brush Border	0(0%)	9(100%)	8(89%)	1(11%)	0(0%)	9(100%)	2(22%)	7(78%)	<0.001*
Epithelial Necrosis	0(0%)	9(100%)	8(89%)	1(11%)	0(0%)	9(100%)	1(11%)	8(89%)	<0.001*
Intraluminal Protein Casts	0(0%)	9(100%)	3(33%)	6(67%)	0(0%)	9(100%)	1(11%)	8(89%)	0.08
Broken Basement Membrane	0(0%)	9(100%)	8(89%)	1(11%)	0(0%)	9(100%)	2(22%)	7(78%)	<0.001*

**Significant p-value*

DISCUSSION

Glutamine has many remarkable functions in the cell, including regulation of cellular redox balance and apoptosis.[9] Gentamicin causes apoptosis in proximal tubule epithelial cells of kidneys in rats.[10] In the present study, histological parameters of groups A, C, and D showed that proximal convoluted tubules and distal convoluted tubules of the kidney were normal. There was no sign of necrosis in the epithelial lining and the basement membrane or brush borders were prominent. In group B, the PCT showed visible necrosis and destruction. The PAS staining of kidney tissue from group B rats showed indistinct PCT with epithelial necrosis, vacuolization, disruption of brush border, presence of intraluminal protein cast, and damage to the basement membrane. Further, in group B the interstitium showed inflammatory zone (IZ) and vascular congestion (VC), which was not observed in the other three groups. Another study reported similar results of kidney damage in all gentamicin-induced groups, which was necrosis in the convoluted tubule and the Bowman's capsule as compared to the control group.[11] A review was conducted by Randjelovic et al. to focus on the current knowledge available regarding gentamicin-induced nephrotoxicity. They reported that gentamicin causes necrosis of tubular epithelial cells, especially in proximal convoluted tubules. It also reduced glomerular filtration leading to the accumulation of proteins and increase excretion of potassium & sodium.[12]

In the current study, we observed epithelial vacuolization, tubular necrosis, and glomerular atrophy in gentamicin-treated animals. Another study reported that intramuscular injection of gentamicin (100 mg/kg/day) in mice for 10 consecutive days can lead to these deleterious effects on kidney structure and function. The cells of necrotic tubules get deprived of their structural and polysaccharide proteins leading to major histological and histochemical alterations in the kidneys resulting in renal failure.[13]

The nephrotoxic effects of gentamicin are largely attributed to disturbances in mitochondrial metabolism which eventually leads to an increase in oxidative stress, which has injurious effects on the cellular function and metabolic integrity of the body. The role of reactive oxygen metabolites (ROS) in gentamicin-induced nephrotoxicity has also been recognized by various other studies.[12,13]

In our study, we observed normal PCT with distinct brush border membrane and DCT in PAS stained sections of kidney tissues in group D animals treated with glutamine. Another study reported similar results when they treated the animals with ficus carica (fig) leaf extract along with gentamicin. They reported that the histological preparations from the gentamicin-positive group showed proximal renal tubular necrosis, degeneration of epithelial cells, and loss of brush border.[13] Further, as compared to the gentamicin-treated group, there was a significant improvement in kidney function and structure in groups that received ficus carica leaf extract along with gentamicin and had nearly normal-looking renal glomeruli with patchy areas of tubular necrosis and reappearance of complete brush borders. These findings also support our results, and it can be concluded that like green tea, ficus carica, leaf extract, and kiwi fruit, glutamine also protects the kidneys from gentamicin toxicity and oxidative damage by improving antioxidant defense, energy metabolism, and tissue integrity.[14] Similarly, the protective effects of other natural compounds including black seeds of nigella sativa (black cumin) and spinach (spinacea oleracea) have also been investigated and have shown to ameliorate the nephrotoxic effects of aspirin and cyclosporin.[15] Another recent study conducted in Iran showed that exogenous glutathione (100 mg/kg intraperitoneally) reduced inflammatory renal changes and improved renal dysfunction in rats with gentamicin-induced acute kidney injury.[9]

CONCLUSION

The nephrotoxicity and renal oxidative damage caused by gentamicin are incredibly significant. Since the effectiveness of gentamicin in the control of various bacterial diseases is quite significant, it is the need of the hour to establish the role of nephroprotective agent with gentamicin administration. The use of glutamine supplements has shown promising results in providing protective effects on kidneys in the presence of gentamicin in rats.

LIMITATIONS & RECOMMENDATIONS

The study has some limitations as the sample size was small and due to inherent time constraints, the duration of the experiment was kept for 7 days. Further studies are needed to standardize the dose of ameliorating compounds so that a reference protocol can be developed for the protection of gentamicin-induced nephrotoxicity. Research should be carried out to explore the dose and dimensions of the use of glutamine and other amino acids as supplements to reduce the negative effects of the drugs that cause oxidative damage and toxicity.

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