AMELIORATIVE EFFECT OF BERBERIS VULGARIS FRUIT EXTRACT AGAINST GENTAMICIN INDUCED NEPHROTOXICITY IN ALBINO RATS

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ABSTRACT

Objective: To observe the nephroprotective role of berberis vulgar is on renal parenchyma against microscopic and morphometric changes induced by Gentamicin in albino rats.

Study Design: Lab based experimental study.

Place and Duration of Study: It was conducted in Baqai Medical University in collaboration with department of Anatomy Liaquat National Hospital and Medical College, from Jan to Jul 2017.

Methodology: A total of 40 male adult albino rats were used in the study. Four groups were made. Each group contained 10 rats. Group A was a control group, group B received only berberis vulgaris fruit extract orally per day for 21 days, group C received Gentamicin 100 mg/kg/day intraperitonially daily for 21 days. Group D received Gentamicin 100 mg/kg/day intraperitonially along with berberis vulgaris fruit extract 100 mg/kg/day orally. Both kidneys were removed. H&E and PAS stains were used for observing histological alterations and protective role of berberis vulgaris fruit extract.

Results: Glomerulus and proximal convoluted tubules were observed histologically in all 4 groups. Microscopy of group B showed parameters nearly similar to control group. Microscopy of group C showed significant derangement in all parameters when compared with control group. Group C showed decrease glomerular, proximal convoluted tubular count was noted. Glomerular diameter increases and there was glomerular hypertrophy and tubular necrosis. Microscopy of group D showed significant improvement due to berberis vulgaris which restored normal renal architecture.

Conclusion: Berberis vulgaris has a nephroprotective effect and it can be used as a new medicine against nephrotoxic drugs like Gentamicin.

Keywords: Berberis vulgaris, Gentamicin, Kidney, Nephrotoxicity.

INTRODUCTION

Kidney play pivot role to remove waste products and overloaded water from the body in the form of urine. Gentamicin is the most common antibiotic against gramnegative bacterial infections. One of the main side effects of gentamicin is nephrotoxicity[1]. Impairment occurs in 30% of patients treated with gentamicin[2].

Gentamicin an aminoglycoside has been reported to induce acute kidney failure due to acute tubular necrosis by increasing serum creatinine and blood urea concentration. This drug also has a potential to cause ototoxicity and nerve damage. Mechanism of Gentamicin induced nephrotoxicity involves different pathways including oxidative stress, inflammation, decreased renal blood flow and increased nitric oxide level (NO)[3]. However Gentamicin play significant role against life threatening infections especially when used in combination therapy[4]. It can lead to renal damage due to tubular necrosis and interstitial damage. Aminoglycosides collect in proximal tubular cells, from where they set apart in lysosomes and communicate with ribosomes, mitochondria and remaining cell organelles to generate cell injury[5]. It works by inhibiting protein synthesis. Berberis vulgaris (Barberry) belongs to the family Berberidaceae. A well-known plant in Iran and has been used widely as a medicinal plant in folk medicine. All parts of berberis vulgaris i.e. root bark, leaves, seeds, and fruits have been used as medicinal pur-pose in Iran and many other countries for long period. Most commonly studied naturally occurring protober-berine alkaloids is berberine[6]. Iran Berberis vulgaris berries commonly called Zereshk. According to World Health Organization (WHO) in about 75-80% of world's population, herbal medicine is widely used as a source for primary health care especially in developing countries. All parts of plant have pharmaceutical properties including anti-inflammatory, antioxidant, antinociceptive, antiarrhythmic, anticholinergic, anti-pruritic, antidiarrheal, antiprotozoal properties[7]. Berberis vulgaris has nephroprotective effect by inhibiting oxidative stress. Berberis act as free radical scavenger and provide strengthful protection in acute kidney damage[8]. Cytoprotective compounds isolated from

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Berberis vulgaris are phenolic compounds which include tyramine, cannabisin G and lyoniresinol. Lyoniresinol has antioxidant properties and cannabisin exhibited cytoprotective activity[9].

METHODOLOGY

Forty healthy male albino rats Sprague Dawley species weighing (180-250) gms 10-12 weeks of age were kept. Rats aged 10-12 weeks and weight between 180-250 gms. All animals were weighed, tagged and kept in separate well ventilated cages of Animal house at the beginning of study. Before the start of experimental procedure all albino rats were kept under observation and acclimatization for 10 days to observe their weight gain or loss, their nutritional status on the basis of their oral intake and excretion, behavioural change and general health conditions. All standarads of laboratory environment were maintained.

Group A rats required no intervention and served as control group. Group B received Berberis vulgaris fruit extract 100 mg/kg/body wt orally per day for 21 days. Group C received Gentamicin 100 mg/kg/day Intraperitonially[10], for 21 days. Group D received Gentamicin 100 mg/kg/day Intraperitonially in a single dose per day along with berberis vulgaris 100 mg kg/day through gastric gavages for 21 days. Rats were anesthetized by ether in a glass container and sacrificed. They were fixed on a dissecting board and abdomen was opened by mid line longitudinal incision that extended from manubrium sternum up to the lower abdomen. Both kidneys were removed and cut into two equal longitudinal halves. General architecture of kidney tissue was observed by staining with haemotoxylin and eosin (H&E) and periodic acid Schiff (PAS) was used to observe brush borders of proximal convoluted tubules, basement membrane intactness, architecture of glomerulus and bowman's capsule under light microscope[11]. Slides of all groups A to D architecture were observed. Random selection of slides was done. In each slides five random fields were selected and in each field almost nearly complete glomerulus and tubule were observed. All slides were observed by light microscope under magnification of 10 and 40.

SPSS version 21 was used for analyzing numerical data. The difference was scrutinized statistically significant when *p*-value was ≤0.05. The differences among treated groups were analyzed by one-way ANOVA.

RESULTS

Renal cortex showed normal architecture of renal corpuscles formed by glomerular tuft of capillaries and bowman's capsules in group A. Basement membrane of glomerulus was intact. The renal columns and medullary rays were alternately arranged. (fig-1). Narrow lumen of proximal convoluted tubule were found. They are present in large number and lined with simple cuboidal cells. Cytoplasm is eosinophilic containing basophilic nuclei close to basement membrane and is spherical in shape. Wide lumen of DCT observed and their numbers also reduced lined with low cuboidal cells, nuclei at the center. Proximal convoluted tubular lumen showed well-demarcated brush border. No cellular debris in the lumen of PCT was observed. Cellular integrity was maintained (fig-1a).

Group B showed no structural change in glomerulus, proximal tubule and loop of henle. The purpose of this group was to find out if berberis vulgaris itself caused any alteration in morphological and histological structure of renal parenchyma (fig-1b).

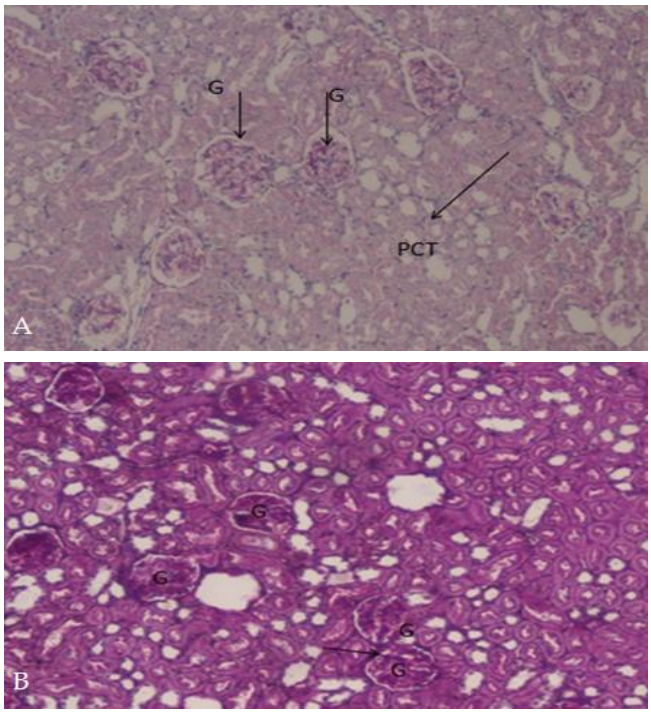

Figure-1: (a) Control group A showing normal glomeruli and pct. H&E stain 100x, (b): Group B showing near normal architecture. PAS stained 100x.

The H & E stained renal sections of group C showed altered architecture of renal parenchyma along with degenerative changes in the renal cortex and medulla. Hypertrophy of glomeruli was observed apparently with congestion of glomerular capallaries, edema and hemorrhage. Renal interstitium showed inflammatory cells with moderate edema and congested and dilated blood vessels (table) (fig-1c).

Histopathology of group D showed significant improvement. The H & E stained sections showed near normal renal architecture with slight congestion and hemorrhage around glomeruli. Minimal changes was observed in the glomeruli and most of the glomeruli showed normal architecture with slight glomerular congestion, peritubular congestion along with blood vessels congestion. It was observed that Berberis vulgaris treated group has restored the normal glomerular structure (fig-1d).

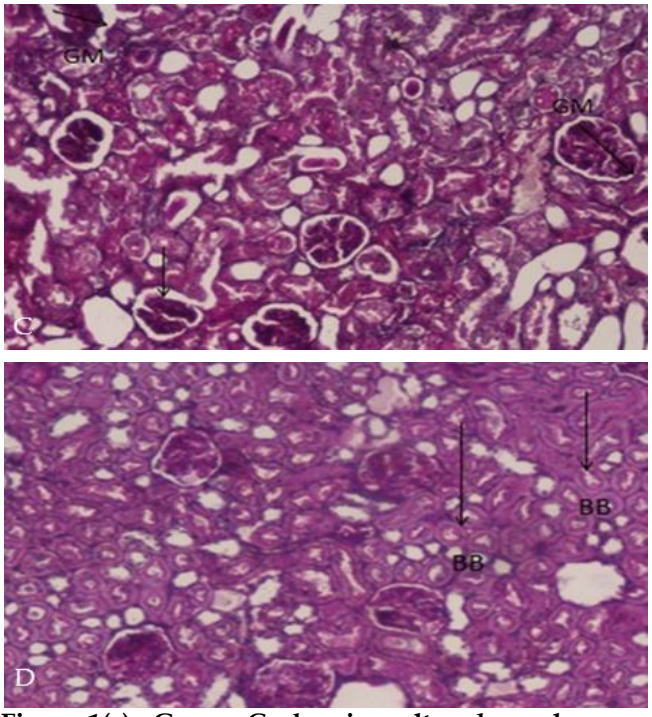

Figure-1(c): Group C showing altered renal parenchyma. Hypertrophy of glomerulus. H & E stain 100x and (d): Group D showing intact basement membrane. PAS stained 100x.

Mean of glomerular count, proximal convoluted tubular count and glomerular diameter of group A animals were 5.73 ± 162, 13.08 ± 0.364 and 61.32 ± 2.18 respectively (fig-2 & 3). Mean of glomerular count, proximal convoluted tubular count and glomerular diameter of group B animals were 5.82 ± 0.98, 12.35 ± 0.185 and 66.4 ± 1.769 respectively (fig 2 & 3). Mean of glomerular count, proximal convoluted tubular count and glomerular diameter of group C animal were 3.76 ± 0.97, 6.15 ± 0.139 and 72.6 ± 0.486 respectively (fig 2 & 3). Mean of glomerular count, proximal convoluted tubular count and glomerular diameter of group D animal were 5.13 ± 0.071, 11.79 ± 0.088 and 65.37 ± 0.7589 respectively (fig-2 & 3).

Table: Mean values (µM) of glomerular count, pct count and glomerular diameter.

Experimental Groups	Control Group	Berberis Only Group	Berberis Treated Group	Gentamicin Only Group	*p*-value
Glomerular Count	5.73 ± 162	5.82 ± 0.98	3.76 ± 0.97	3.76 ± 0.97	0.0001
Pct Count	13.08 ± 0.364	12.35 ± 0.185	6.15 ± 0.139	6.15 ± 0.139	0.0001
Glomerular Diameter	61.32 ± 2.18	66.4 ± 1 0.769	72.6 ± 0.486	72.6 ± 0.486	0.0001

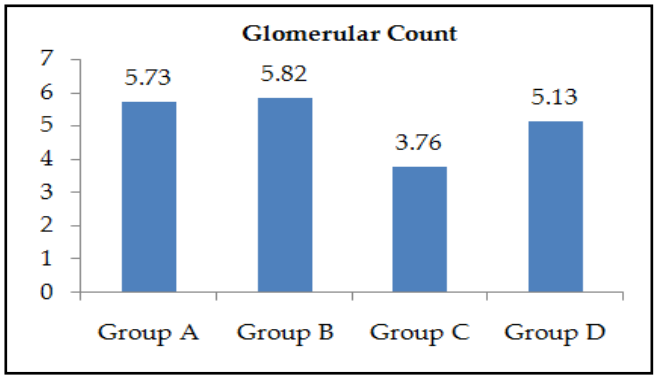

Figure-2a: Comparision of mean values of glomerular count in different groups of experiment.

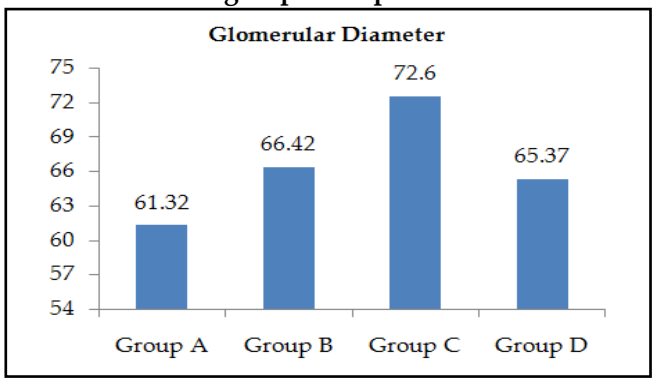

Figure-2b: Comparision of mean values of glomerular diameter in different groups of experiment.

DISCUSSION

Gentamicin is the commonly used antibiotic especially in developing counteries because of its low cost and easy availability, however it can induce nephrotoxicity. We observed the effects of gentamicin and berberis vulgaris on glomerulus and proximal convoluted tubules. It was noted in this study that there was decrease in the number of glomerular count, PCT count and Glomerular diameter in groups treated

with gentamicin which was due to decrease in body weight and kidney weight. Body weight is the most precise indicator showing adverse effects of different xenobiotics. Difference of weight gain is due to catabolic activity which occurs as a result of renal dysfunction due to gentamicin , leading to acidosis and is accompanied by anorexia, food intake diminishes results in body weight loss due to gentamicin Generation of ROS may lead to cell damage as well as cell death by scattered mechanism e.g.formation of ATP, cytochrome c release, lipid peroxidation, DNA damage by cell cycle arrest, all these results in cell swelling and necrosis[12,13].

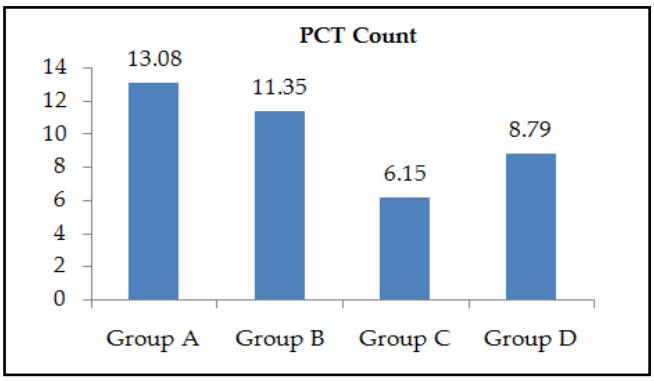

Figure-3: Comparision of mean values of proximal tubular count in different groups of experiment.

Berberis vulgaris protected group D rats were as active as control group. This is due to the fact that berberis vulgaris prevents oxidative damage and induces antioxidant enzymatic activities by decreasing free radicals generation. Therefore berberis vulgaris has antioxidantal properties which boost health, hence increased body weight[14].

Aminoglycosides with the help of endocytosis entered into the proximal tubular endothelial cells of kidney and remain there for a certain time which generates nephrotoxicity[15,16]. Brush border of proximal tubular renal cells contain acidic phospholipids, also present in plasma membrane of different tissues, were considered as a binding site for aminoglycoside. Pathogenesis of aminoglycosides nephrotoxicity includes suppresssion of the activity of Na ± K ± ATPase and stops synthesis of DNA in proximal tubules of albino rats causing renal injury[17]. Death of proximal tubular cells occurred by gentamicin because of oxidative stress, apoptosis and inflammatory process[18]. It was described by Quiros *et al* that gentamicin strongly affects proximal tubules and does not produce alteration in distal tubules[19,20].

Nephrotoxicity induced by gentamicin also involves lipid peroxidation. Gentamicin induced nephrotoxicity is structurally evident by glomerular hypertrophy or atrophy, tubular necrosis, haemorrhage and edema[21,22]. One of the main mechanism of nephrotoxicity is oxidative stress generated by gentamicin drug which impaired renal function. Berberis vulgaris prevents oxidative damage and induces antioxidant enzymatic activities by decreasing free radicals generation. Berberis vulgaris extract react with metabolites of lipid peroxidation and free radicals as well, thereby increase thiol contents of tissue and produces antioxidant activities. Antioxidants can reverse most of the alterations in histological sections of renal tissue generated by gentamicin. Group B berberis treated group showed similar findings when compared with control group. Group gentamicin treated with berberis showed mild proximal tubular necrosis which showed that berberi vulgaris neutralized nephrotoxicity induced by gentamicin. Similar findings were also reported by chinnapa reddy *et al*[23].

Gentamicin treated group produced alteration in glomerular structure in the form of mesangial hypercellularity and proliferation of endothelial cells of glomerulus. There was decrease in glomerular count due to inflammatory process as well in this study. This finding was according to the previously done studies by Alarifi *et al*[24].

Microscopic examination of gentamicin treated group C of renal tissues showed changed parenchyma of kidney with proximal tubular dilatation, loss of brush border and necrosis of proximal tubular cells. Dilated proximal tubules contained degenerative and desquamative type cells. It was noted that presence of cytoplasmic vacuoles are due to toxic effect of gentamicin that is dilatation and destruction of the organelles. Most badly affected area by gentamicin is proximal convoluted tubules. It was found that berberis vulgaris possess nephroprotective activity (Soliman *et al*, 2007)[25].

CONCLUSION

It is concluded that Gentamicin produces oxidative nephrotoxic effects on renal parenchyma which can be limited and prevented by protective antioxidative effect of Berberis vulgaris that restored histomorphological changes in renal tissue induced by gentamicin toxicity.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

REFERENCES

1. Ahmadvand H, Tavafi M, Asadollahi V, Jafaripour L, Hadipour-Moradi F, Mohammadrezaei-Khoramabadi R, et al. Protective effect of carvacrol on renal functional and histopathological changes in gentamicin-induced-nephrotoxicity in rats. Zahedan J Res Med Scien 2016; 18(4): 1-5.
2. Adil M, Kandhare AD, Dalvi G, Ghosh P, Venkata S, Raygude KS, et al. Ameliorative effect of berberine against gentamicin-induced nephrotoxicity in rats via attenuation of oxidative stress, inflammation, apoptosis and mitochondrial dysfunction. Renal failure 2016; 38(6): 996-06.
3. Morsy MA, Ibrahim SA, Amin EF, Kamel MY, Rifaai RA, Hassan MK. Sildenafil ameliorates gentamicin-induced nephrotoxicity in rats: role of iNOS and eNOS. J Toxicol 2014; 2014(1): 489382-85.
4. Awodele O, Tomoye OP, Quashie NB, Amagon KI. Gentamicin nephrotoxicity: Animal experimental correlate with human pharmacovigilance outcome. Biomed J 2015; 38(1): 125-30.
5. Veljković M, Pavlović DR, Stojiljković N, Ilić S, Jovanović I, Poklar Ulrih N, et al. Bilberry: chemical profiling, in vitro and in vivo antioxidant activity and nephroprotective effect against gentamicin toxicity in rats. Phytoth Res 2017; 31(1): 115-23.
6. Imanshahidi M, Hosseinzadeh H. Pharmacological and therapeutic effects of Berberis vulgaris and its active constituent, berberine. Phytoth Res 2008; 22(8): 999-12
7. Mokhber-Dezfuli N, Saeidnia S, Gohari AR, Kurepaz-Mahmood abadi M. Phytochemistry and pharmacology of berberis species. Pharmacog Rev 2014; 8(15): 8-10.
8. Domitrović R, Cvijanović O, Pernjak-Pugel E, Škoda M, Mikelić L, Crnčević-Orlić Z. Berberine exerts nephroprotective effect against cisplatin-induced kidney damage through inhibition of oxidative/nitrosative stress, inflammation, autophagy and apoptosis. Food Chemical Toxicol 2013; 62(1): 397-06.
9. Tomosaka H, Chin YW, Salim AA, Keller WJ, Chai H, Kinghorn AD. Antioxidant and cytoprotective compounds from Berberis vulgaris (barberry). Phytoth Res 2008; 22(7): 979-81.
10. Patil CR, Jadhav RB, Singh PK, Mundada S, Patil PR. Protective effect of oleanolic acid on gentamicin induced nephrotoxicity in rats. Phytoth Res 2010; 24(1): 33-37.
11. Bancroft JD, Layton C. Connective and mesenchymal tissues with their stains. Bancroft's theory and practice of histological techniques, expert consult: online and print, 7: Bancroft's Theory and Practice of Histological Techniques. Avalibal At Internet 2013. https://www. elsevier.com/books/bancrofts-theory-and-practice-of-histological-techniques/unknown/978-0-7020-6886-7
12. Sawardekar SB, Patel TC. Evaluation of the effect of Boerhavia diffusa on gentamicin-induced nephrotoxicity in rats. J Ayurveda Integ Med 2015; 6(2): 95-98.
13. Erdem A, Gündogan NÜ, Usubütün A, Kılınç K, Erdem ŞR, Kara A, et al. The protective effect of taurine against gentamicin induced acute tubular necrosis in rats. Nephrol Dialysis Transplantat 2000; 15(8): 1175-82.
14. Laamech J, El-Hilaly J, Fetoui H, Chtourou Y, Tahraoui A, Lyoussi B. Nephroprotective effects of berberis vulgaris l. total extract on lead acetate-induced toxicity in mice. Ind J Pharmaceut Sci 2016; 78(3): 326-33.
15. Padmini MP, Kumar JV. A histopathological study on gentamycin induced nephrotoxicity in experimental Albino rats. IOSR J Dent Med Sci 2012; 1(1): 14-17.
16. Romero F, Pérez M, Chávez M, Parra G, Durante P. Effect of uric acid on gentamicin induced nephrotoxicity in rats–role of matrix metalloproteinases 2 and 9. Basic Clinical Pharmacol Toxicol 2009; 105(6): 416-24.
17. Kadkhodaee M. Erythropoietin; bright future and new hopes for an old drug. J Nephropathol 2012; 1(2): 81-85.
18. Tavafi M, Ahmadvand H, Toolabi P. Inhibitory effect of olive leaf extract on gentamicin-induced nephrotoxicity in rats. Iran J Kidney Dis 2012; 6(1): 25-28.
19. Moghadam A, Khozani TT, Mafi A, Namavar MR. Effects of platelet-rich plasma on kidney regeneration in gentamicin-induced nephrotoxicity. J Korean Med Sci 2017; 32(1): 13-21.
20. Havasi A, Borkan SC. Apoptosis and acute kidney injury. Kidney Inter 2011; 80(1): 29-40.
21. Sodimbaku V, Pujari L, Mullangi R, Marri S. Carrot (Daucus carota L.): Nephroprotective against gentamicin-induced nephrotoxicity in rats. Ind J Pharmacol 2016; 48(2): 122-25.
22. Tavafi M. Inhibition of gentamicin–induced renal tubular cell necrosis. J Nephropathol 2012; 1(2): 83-85.
23. Reddy VC, Amulya V, Lakshmi C, Reddy K, Praveen D, Pratima D, et al. Effect of simvastatin in gentamicin-induced nephrotoxicity in albino rats. Asian J Pharm Clin Res 2012; 5(1): 36-40.
24. Alarifi S, Al-Doaiss A, Alkahtani S, Al-Farraj S, Al-Eissa MS, Al-Dahmash B, et al. Blood chemical changes and renal histological alterations induced by gentamicin in rats. Saudi J Biological Sci 2012; 19(1): 103-10.
25. Soliman KM, Abdul-Hamid M, Othman AI. Effect of carnosine on gentamicin-induced nephrotoxicity. Medical Sci Monitor 2007; 13(3): BR73-BR83.