Nephrotoxic effects of *Valeriana wallichii*

Naveed Ullah[1*], Mir Azam Khan[2], Abdur Rauf[3], Taous Khan[4], Ghulam Murtaza[4], Salimullah Khan[5] and Ismail Shah[5]

[1]Department of Pharmacy, University of Swabi, Swabi, Pakistan
[2]Department of Pharmacy, University of Malakand, Chakdara, Pakistan
[3]Department of Chemistry, University of Swabi, Swabi, Pakistan
[4]Department of Pharmacy, COMSATS Institute of Information Technology, Abbottabad, Pakistan
[5]Department of Pharmacy, Abdul Wali Khan University, Mardan, Pakistan

Abstract: Aminoglycosides are the commonly used antibiotics against Gram negative bacteria. Their clinical applications are limited due to nephrotoxic side effects. Therefore, the current study was undertaken in an attempt to increase the use of these drugs without causing nephrotoxicity by exploring the nephroprotective effects of a medicinal plant with high flavonoid contents and strong antioxidant properties, namely *Valeriana wallichii*. A daily dose of 200mg/kg of the extract derived from *V. wallichii* was employed for a period of three weeks. The results obtained revealed that co-therapy of extract with gentamicin protected some changes in renal functions; however, failed to provide a complete protection as assessed by biochemical, physiological and histological parameters. It can be concluded from the current findings that *V. wallichii* failed to deliver protective effects against gentamicin induced renal damage in spite of strong flavonoid contents and antioxidant properties.

Keywords: *Valeriana wallichii*, nephrotoxicity, aminoglycosides, flavonoids, antioxidant.

INTRODUCTION

Aminoglycosidesare bactericidal drugs, which are effective against Gram negative microorganisms (Jawetz, 1992). Despite of low chances of bacterial resistance (Chow *et al*., 1991), their clinical applications are limited because of renal damaging side effects. If the matter of gentamicin nephrotoxicity is managed then there would be no need for development of any more drugs against Gram negative microorganisms (Gilbert and Bennett, 1993). Currently, there is an increasing trend in the use of herbal medicines for the treatment of different ailments; therefore, the pharmacological screening of medicinal plants has got more attention recently.

Valeriana wallichii DC (Valerianaceae) is a small perennial herb having sharply pointed leaves and clustered white and pink flowers. It is mostly found in Northern areas of Pakistan (Baquar, 1989). It is commonly used for the treatment of scorpion stings, jaundice, insomnia, neurosis, epilepsy and sciatica (Marder, *et al*., 2003). The plant has been reported with a number of medicinal properties including analgesic (Vohora and Dandiya, 1992), cytotoxic (Khuda *et al*., 2012), antispasmodic and hypotensive (Gilani *et al*., 2005), anxiolytic and antidepressant (Ron *et al*., 2000) and have no toxic effect (Joseph *et al.*, 2015). In some countries, it is used for the treatment of habitual constipation (Baquar, 1989) and its herbal preparation is effective in dyspepsia (Tripathi *et al*., 1982). Moreover, the ethanolic extract of *Valeriana wallichii* has been reported with strong dose dependent partial hepatoprotection against CCl4 induced toxicity (Syed *et al*., 2014). The chemical study of the plant has shown that it contains a high flavonoid content (6-methylapigenin and hesperidin) and has a strong antioxidant properties (Marder *et al*., 2003; Niezen *et al*., 1995; Bate-Smit, 1962; Subhan *et al*., 2007). The essential oil obtained from this plant has been reported to have antibacterial and antifungal properties (Suri and Thind, 1978). Furthermore, the valeric acid present in *V. wallichii* has been reported with significant GABAergic effect in amelioration of experimental dementia (Vishwakarma *et al*., 2016).

According to the above discussed literature, *V. wallichii* possesses a high flavonoid content and strong antioxidant properties, which may facilitate the inhibition of oxidative cellular damage and thus may protect against gentamicin induced renal injury. Based on this hypothesis, the present study was aimed to investigate the nephroprotective role of *V. wallichii* against gentamicin induced toxicity.

MATERIALS AND METHODS

Plant material and extraction

Sufficient quantity of *V. wallichii* rhizomes were collected from northern areas of Pakistan in December, 2010. The identification of plant was confirmed by Prof. Umar Farooq, Department of Botany, Government College Abbottabad, Pakistan. The voucher specimen (1026) was deposited in the herbarium of the same institution. The rhizomes were chopped, shade dried and powdered. The powdered plant material was extracted with sufficient amount of 70% aqueous ethanol for three weeks with

**Corresponding author:* e-mail: naveedjia@yahoo.com

occasional shaking. The extract was filtered and concentrated under reduced pressure in a rotary evaporator (R-210 Germany).

Study design

Animals handling and care were strictly performed according to the guidelines of the University (University of Malakand) along with international laws and policies (National Institutes of Health Guide for the Care and Use of Laboratory Animals, NIH Publication no. 85-23, 1985) after the approval of project from University Research Committee. Twenty four male rabbits (1-1.5kg) of local breed were used in this study. Animals were maintained on same diet for fifteen days before the start of study. They were divided in four groups each containing six rabbits. The extract was provided orally while gentamicin was administered intramuscularly for twenty one days according to the dosage regimen given in table 1.

Samples collection

Blood and urine samples were collected thrice in the experimental period i.e., on day 0, 11 and 21. Serum was separated from the blood with the help of micropipette for estimation of urea, creatinine, uric acid, sodium, calcium and potassium. Twenty four hours urinary volume was measured with a graduated cylinder. The estimation of urinary urea, creatinine, proteins and enzymes were performed by using fresh samples of urine. The weight of each animal was measured on day 0, 11, and 21. On the last day of experimental period, kidneys were isolated for histological examination.

Histopathological examination of kidney

One kidney of each animal was cut down longitudinally while the other transversely and fixed in 10% formalin solution. The tissues were then dehydrated with the ascending grades of alcohol from 50-90%. Finally, the absolute alcohol was applied followed by xylene. The tissues were then imbedded in paraffin wax. The solidified blocks were cut down into a number of thin pieces with the help of Rotatory microtome (Micros, Germany). The tissues were stained with hematoxylin and eosin dyes and studied under light microscope (Germany).

Measurement of blood urea nitrogen (BUN) and creatinine

BUN was measured by using modified Bertholot's indophenol assay (Smith, 1985) while serum creatinine was measured by following Jaffe reaction (smith, 1985). ProDia reagent kits were used for the estimation of both BUN and creatinine with the help of a Power lab-300 (Merck, Germany).

Measurement of serum uric acid and creatinine clearance

Commercially available reagent kits were used for measurement of serum uric acid with the help of Chemistry analyzer power lab 300 (Merck, Germany), while creatinine clearance was calculated from serum creatinine and urinary creatinine by applying the formula;

$$Creatinineclearance = \frac{\text{Urinary Creatinine Concentration}}{Serumcreatinineconcentration} \times \text{Urinary Volume}$$

Measurement of serum electrolytes

Serum calcium was measured by cresolphthalein complexone method (Blosser, 1985) by using commercially available reagent kits (Randox Lab, UK), while serum sodium and potassium was measured with flame photometer(PFP-7, England) (Blosser, 1985).

Measurement of urinary proteins and enzymes

Urinary total protein was measured by using commercially available kits (DiaSys Diagnostic, Germany) (Johnson *et al*., 1999), while urinary alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were determined according to the German Society of Clinical Chemistry (Deutsche, 1972).

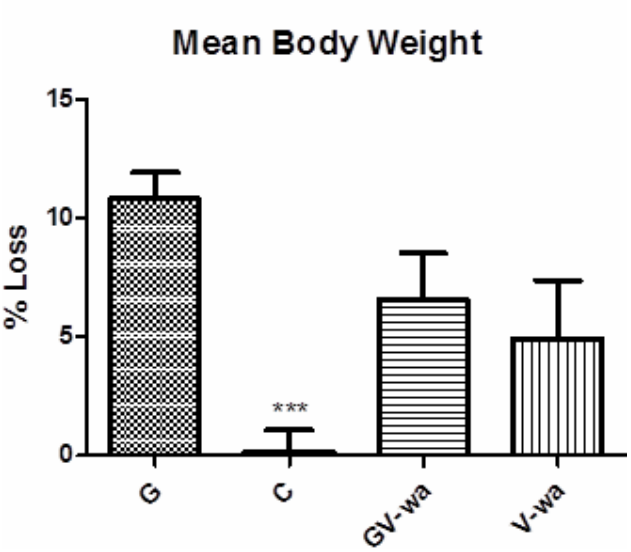

Fig. 1: Percent loss in mean body weight in various experimental groups.

Urinary analysis by reagent strips and microscopy

URS-10 reagent strips (Teco Diagnostic) were used for the identification of urinary glucose, ketones, specific gravity, bilirubin, pH, proteins, RBC, WBC, urobilinogen and nitrite. For further confirmation of renal damage, a drop of urine after centrifugation was examined under microscope (Germany).

STATISTICAL ANALYSIS

The mean of each group was compared with the gentamicin treated groups by one way analysis of variance followed by Dunnett-test, and the results were expressed as mean ± standard error. The difference between two groups was considered significant when the *p* value was <0.05.

RESULTS

Body weight

A decrease in the mean body weight was observed in all selected groups; the control group lost 0.155±0.91% of their initial body weight while gentamicin treated animals lost 10.795±1.09% of their body weight. Group GV-wa and V-wa animals lost 6.53±1.97% and 4.89±2.43%, respectively of their body weights. The difference in the weight loss of group GV-wa and V-wa was not significant when compared with gentamicin treated group, however, control group was found significantly different when compared with group G animals (fig. 1).

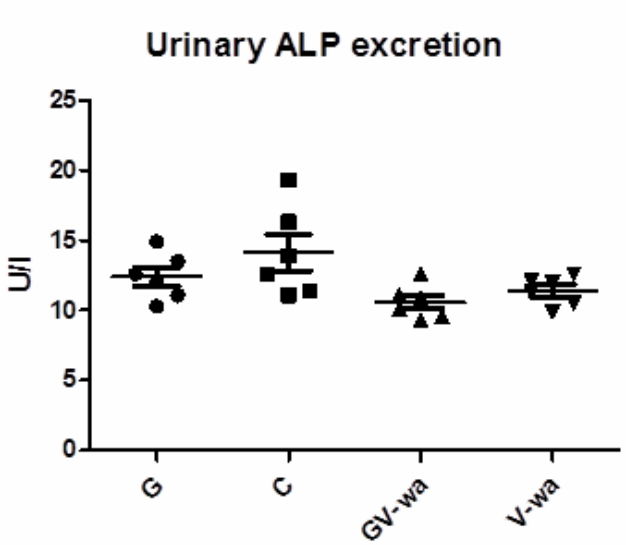

Fig. 2: ALP excretion on day 21 of study period in various groups.

BUN

Progressive rise in BUN was observed in all treated groups but this rise in group G was found extremely significant when compared with other three groups. The increase in BUN on the last day of experimental period in group G animals was measured as 54.18±2.6mg/dl that was statistically different from control (14.14±1.12mg/dl), GV-wa (27.43±3.42mg/dl) and V-wa (22.60±0.79mg/dl), respectively.

Serum creatinine

An increasing trend was also observed for serum creatinine level in all treated groups including group C, group G, group GV-wa and group V-wa. The serum creatinine level in group GV-wa, V-wa and control group were found significantly different when compared with group G (2.87±0.14mg/dl, 1.12±0.05mg/dl and 0.80±0.10 mg/dl vs group G 4.02±0.14 mg/dl).

Creatinine clearance

Creatinine clearance fall significantly in group G animals when compared with control i.e., 0.76±0.09 ml/min vs group C 4.99±1.16 ml/min. The difference in creatinine clearance of group GV-wa (1.04±0.09 ml/min) was not found significant when compared with group G.

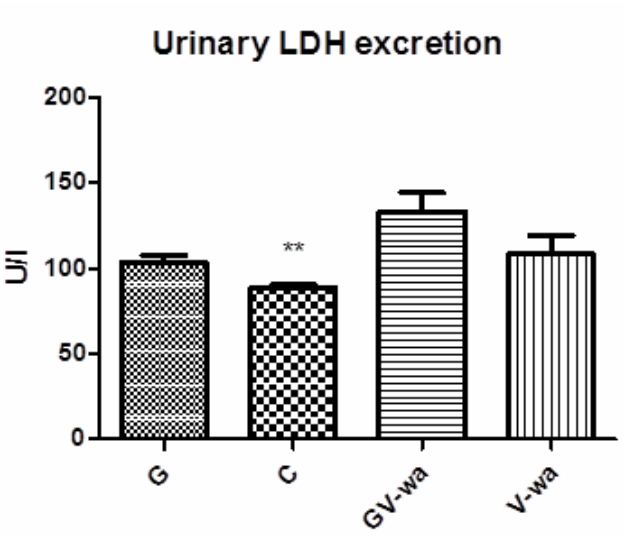

Fig. 3: LDH excretion on day 21 of study period in various groups.

Serum uric acid

Serum uric acid level was raised in group G (2.34±0.12mg/dl) and was found significantly different when compared with group V-wa (1.74±0.19mg/dl) and extremely significant when compared with control group (1.51±0.02mg/dl). No significant difference was observed when compared with group GV-wa (2.40±0.14mg/dl) as given in table 2.

Serum electrolytes

No significant difference was observed in the serum sodium level of any group throughout the study period as given in table 2. On the other hand, severe hypokalemia was noted in group G animals (3.43±0.17mEq/l) when compared with control (5.10±0.24mEq/l). A significant difference was observed in group V-wa and group G, however, the difference was not significant when group GV-wa was compared with group G animals as given in table 2. Furthermore, on the last day of study period, a significant fall in serum calcium was noted in group G animals (7.68±0.21mg/dl) compared to control (9.72± 0.25mg/dl). These results were significantly different from group V-wa while extremely significant when compared with GV-wa.

Urinary proteins

Extremely significant increase in urinary protein excretion was observed in group G (3.86±0.32mg/dl) and GV-wa (4.10±0.31mg/dl) vs controls (1.81±0.22mg/dl) while group V-wa (2.18±0.24mg/dl) was found significantly different when compared with group G.

Measurement of urinary volume

Significant fall in the urinary volume was observed in group G (126±9.09 ml), GV-wa (148±18.21 ml) and V-wa

(136±17.86 ml) animals when compared with control (217±19.77ml) as presented in table 2.

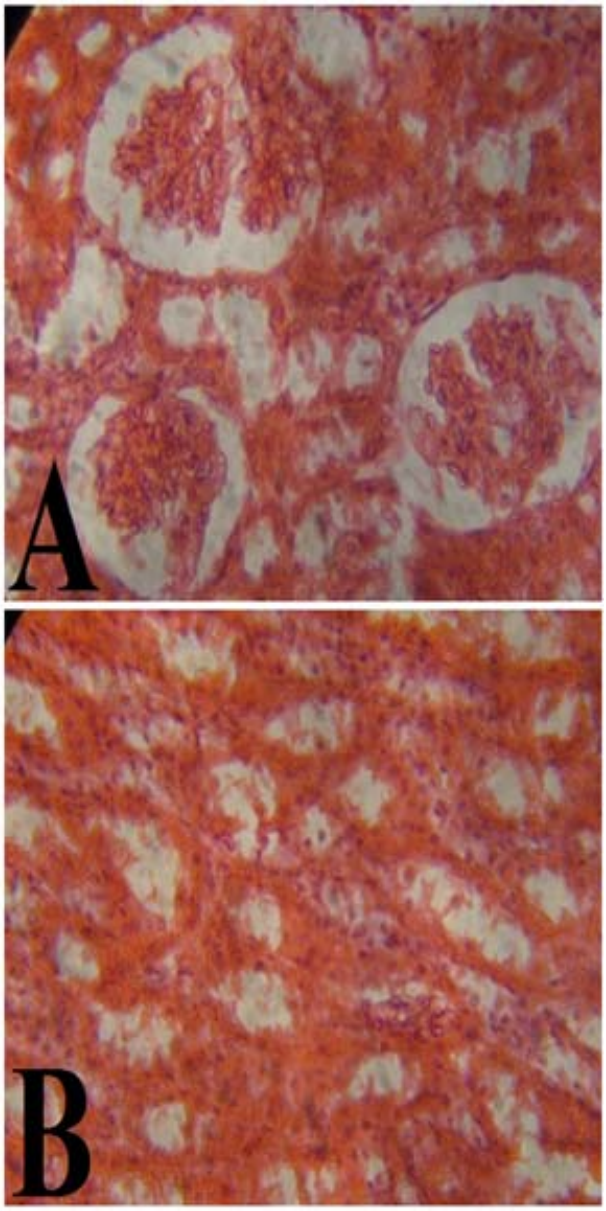

Fig. 4: Microphotographs of Control group: (A) Cortex presenting normal glomerular structures (B) Medulla presenting normal tubules but with hazy look.

ALP excretion

No significant change was observed in the urinary excretion of ALP in group C, GV-wa and V-wa when compared with group G throughout the experimental period as shown in fig. 2.

LDH excretion

Initially on day 11 of study period, extremely significant rise in urinary LDH was observed in group G (143.17±3.53U/l) vs control (91.33±1.86U/l). This further decreased to 103.17±4.28 U/l on day 21 but was still significant when compared with the control (88.17±2.24 U/l). Group GV-wa and V-wa were found significantly different from group G on day 11 while on day 21, group GV-wa was observed to have no significant difference from group G (fig. 3).

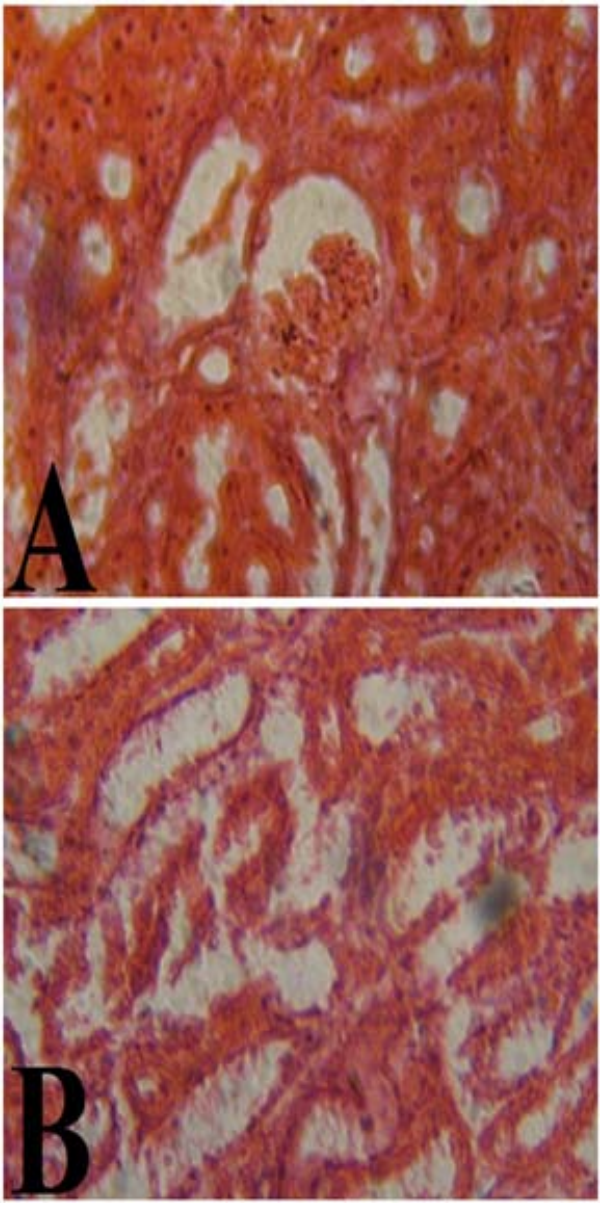

Fig. 5: Microphotographs of Gentamicin treated group: (A) Cortex showing atropic glomeruli with some hydropic abnormalities. Cellular pattern was seen to lost, presenting necrosis and hyaline filled lumina (B) Medulla showing ruptured and dilated tubules with loss of cellular pattern.

Urinary examination by microscope

Renal casts and white blood cells were abundantly diagnosed in group G, group GV-wa and V-wa animals. However, calcium oxalate and yeast cells were not detected up to significant level. Further, no significant abnormality was noticed in the urine of control group animals.

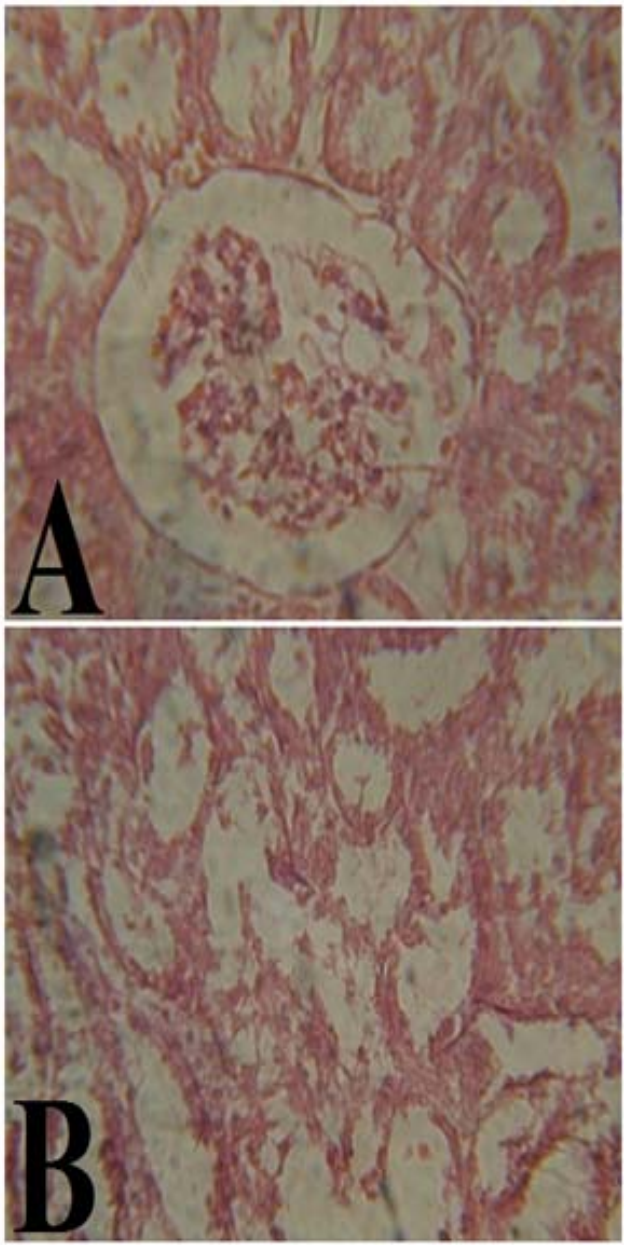

Fig. 6: Microphotographs of Group GV-wa: (A) Cortex presenting ruptured and hyaline filled tubules with hydropic changes (B) Medulla presenting large number of dilated ruptured tubules with renal casts

Urinary examination by reagent strip

Trace amount of proteins, leukocytes and red blood cells were noted in group G and GV-wa, however, no significant changes were detected in group V-wa and control group animals. Glucose and ketones were not detected in any group on last day of experimental period. Mild to moderate amount of bilirubin was also detected in all experimental groups. Foully smell was noted in the urine of group G and GV-wa animals. The pH of the urine was recorded to be basic throughout the study period.

Histopathological investigations

Group G and GV-wa animals after treatment showed increased cellularity and atropy of most of the glomeruli (fig. 5A, 6A). However, no hazy appearance with normal glomeruli was observed in group C. Necrosis of the proximal tubular cells was found in group G animals whose cellular pattern was disturbed (fig. 5A).Normal cellular pattern with no necrosis was observed in control group animals (fig. 4A, 4B). Hyaline filled lumina with granular cast were also identified in group G and GV-wa (fig. 5A, 5B, 6A). Ruptured tubules were detected and most of the tubular cells were found flattened with loss of their proper structures (fig. 5B, 6A, 6B). Hydropic changes in the tubules were observed in group G, GV-wa and V-wa animals (fig. 5A, 5B, 7B). Collecting tubules were diagnosed to be dilated in the medulla of group G and GV-wa animals with few renal casts (fig. 5B, 6B).

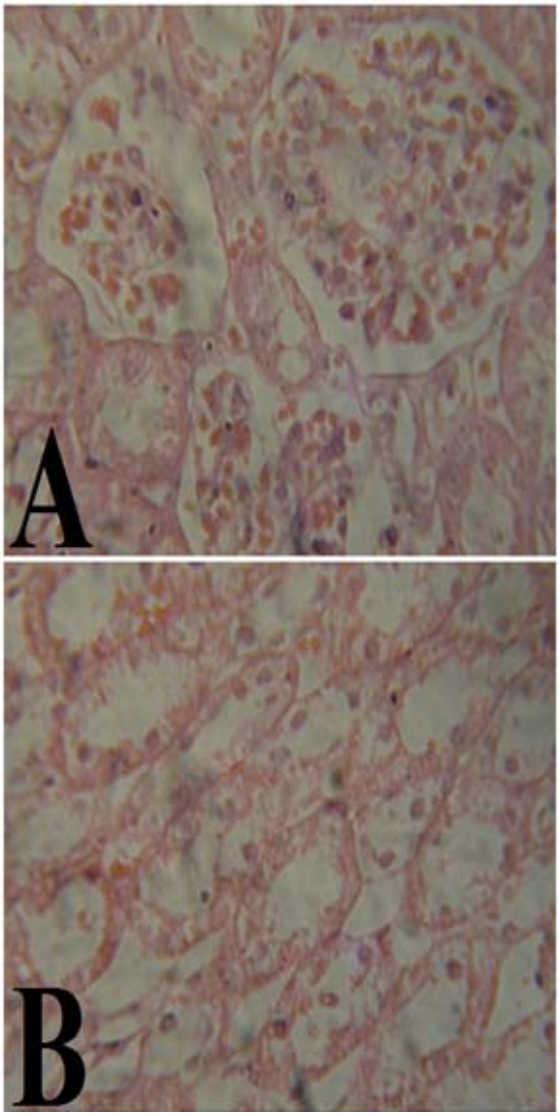

Fig. 7: Microphotographs of Group V-wa: (A) Cortex presenting normal glomeruli and tubules (B) Medulla presenting normal tubular structures with no evidence of necrosis.

DISCUSSION

The present study was conducted to explore the protective potentials of *V. wallichii* against gentamicin induced renal toxicity. According to Tulkens, (1989) and Bennett *et al*., (1991); functional and morphological changes in the kidney is only observed when gentamicin is taken five to ten times more than normal doses. Additionally it has also been reported that daily dose of 30-60mg/kg of gentamicin for 5-10 days causes necrosis of proximal tubular cells with the association of increased serum creatinine level (Tulkens, 1989). Different researchers used different doses of gentamicin to produce nephrotoxicity, mostly as 40mg/kg/day and 60mg/kg/day (Gilbert *et al.*, 1989; Zager, 1992). A daily dose of 80mg/kg/day of gentamicin was used in the present study to produce a significant nephrotoxic effects.

Bennett *et al*., (1991) reported that gentamicin produces increase in the BUN and serum creatinine with a significant decrease in creatinine clearance. Similarly, in the present study extremely significant increase in BUN, serum creatinine and serum Uric acid level was observed when compared with control group animals. The difference in BUN and serum creatinine of group GV-wa and V-wa was extremely significant when compared with toxic group. However, no significant difference was noted in creatinine clearance of group GV-wa and group G.

Hypokalemia associated with gentamicin has been reported in several studies (Brinker *et al*., 1981; Bennett *et al*., 1991; Thompson *et al*., 1990, Cronin *et al*., 1980). The results obtained in the current study also showed hypokalemia in group G and GV-wa, which is agreement with the previous reports. This may be due to the gentamicin induced depression of sodium-potassium ATPase (Cronin *et al*., 1982). Significant loss in the mean body weight in group G, GV-wa and V-wa provides a statement of loss of potassium (Brinker *et al*., 1981).

Cronin *et al*., (1980) reported a significant fall in serum calcium level of gentamicin treated animals, similar observations was noted in the present study in gentamicin treated group which was extremely different when compared with control and GV-wa groups. Further, Brinker *et al*., (1981) reported gentamicin to have no significant effect on serum calcium level and Bennett *et al*., (1991) presented abnormal excretion of sodium, calcium, potassium and magnesium in the urine of gentamicin treated animals. However, in the current study no significant change in the serum sodium level of any group was observed.

Researchers have presented a number of mechanisms regards the rise of serum creatinine and glomerular functional changes. A relation between serum creatinine and tubular necrosis was reported by Solez (1983). It is due to the obstruction of tubules either by leaking of filtrate from ruptured tubules or by necrotic debris, which may be responsible for the elevation of serum creatinine due to reduce excretion. In the current study urinary protein excretion was found elevated on the last day of experimental period in group G and GV-wa animals, which was extremely different from control group animals. Further, urinary volume was found significantly decreased in group G, GV-wa and V-wa when compared with control group animals. Tubular brush border enzymes were also evaluated by a number of researchers to study renal damage (Fruta and Nakada, 1993). Similarly, in the present work we also studied urinary measurement of LDH and ALP to confirm theeffects of extracts and gentamicin on kidney functioning. ALP remained the same throughout the study period in all experimental groups while LDH excretion increased in group G on day 11 in comparison with other groups, which showed acute renal damage. Further on day 21 urinary excretion of LDH decreased, which provided a statement for early detection of nephrotoxicity. It can be concluded that estimation of urinary enzymes excretion for evaluation of nephrotoxicity is only important for diagnosing early damage.

The histological examination revealed the presence of regenerating cells in the medullary tubules of group GV-wa, indicating that both regeneration and necrosis are simultaneously present neutralizing each other. These results are similar to those presented by Bennett *et al*., (1991) and Cronin *et al*., (1980). In the current study, hyaline filled lumina with granular cast were detected in the tubules of group G and GV-wa, which may cause leaking of proteins causing obstruction that cause a decreased glomerular filtration rate which in turn may be responsible for renal damage (Solez, 1983). Further, animals treated with only extract of *V. wallichii* produced vacuoles in the cells of proximal tubules with normal kidney functions, and these vacuoles are either due to pinocytotic activity or may be due to proteins and salts. The mechanism being responsible is not clear but not associated with substantial cell damage (Gilbert *et al*., 1989; Thompson *et al*., 1990).

Daily dose of 200mg/kg of *V. wallichii* protect changes in BUN, serum creatinine and serum calcium associated with gentamicin but it failed to defend alteration in creatinine clearance, serum uric acid, urinary proteins excretion, urinary volume and urinary LDH excretion associated with gentamicin. From this it can be concluded that *V. wallichii* failed to protect renal damage associated with gentamicin assessed by a number of kidney functioning parameters and histopathological examination.

Table 1: Dosage regimen for twenty one days of experimental period

S. No.	Group	Received
1	C	2ml/kg/day 0.9% saline water
2	G	80mg/kg/day gentamicin
3	GV-wa	80mg/kg/day Gentamicin + 200mg/kg/day *V. wallichii*
4	V-wa	200mg/kg/day *V. wallichii*

Table 2: Various kidney functioning parameters on day 0, 11 and 21 of study period

Parameter	Day	Group C	Group G	Group GV-wa	Group V-wa
Serum BUN (mg/dl)	0	13.05±1.15	12.82±1.11	13.11±1.16	14.06±0.41
	11	13.75±1.04***	37.78±2.14	21.28±2.54***	18.46±0.70***
	21	14.14±1.12***	54.18±2.60	27.43±3.42***	22.60±0.79***
Serum creatinine (mg/dl)	0	0.68±0.07	0.52±0.03	0.66±0.06	0.67±0.07
	11	0.71±0.10***	1.96±0.14	1.22±0.15**	1.06±0.08***
	21	0.80±0.10***	4.02±0.14	2.87±0.14***	1.12±0.05***
Creatinine clearance (ml/min)	0	5.65±0.63	5.28±0.46	5.02±0.33	5.90±0.41
	11	5.08±0.82*	2.08±0.25	2.72±0.44	3.06±0.28
	21	4.99±1.16***	0.76±0.09	1.04±0.09	2.78±0.34**
Serum Uric acid (mg/dl)	0	1.23±0.07	1.11±0.08	1.21±0.09	1.16±0.08
	11	1.39±0.04	1.56±0.05	1.91±0.22	1.42±0.17
	21	1.51±0.02***	2.34±0.12	2.40±0.14	1.74±0.19*
Serum sodium (mEq/l)	0	140.5±1.20	141.17±0.75	140.83±0.98	140.5±1.52
	11	139.6±0.56	140.5±0.56	139.83±0.87	139.5±1.11
	21	140.17±1.01	137.67±1.09	139.16±0.91	139.16±1.19
Serum Potassium (mEq/l)	0	5.30±0.19	5.21±0.21	5.40±0.25	5.01±0.48
	11	5.26±0.15**	3.96±0.14	4.65±0.31	4.36±0.33
	21	5.10±0.24***	3.43±0.17	4.16±0.36	4.20±0.30
Serum Calcium (mg/dl)	0	10.12±0.16	10.28±0.29	10.11±0.09	10.06±0.23
	11	9.96±0.17***	8.48±0.34	10.03±0.08***	8.60±0.28
	21	9.72±0.25***	7.68±0.21	9.23±0.24**	7.11±0.13
Urinary Protien (mg/dl)	0	1.98±0.25	1.56±0.20	1.81±0.33	1.83±0.16
	11	1.64±0.17*	2.51±0.30	2.96±0.30	1.94±0.15
	21	1.81±0.22***	3.86±0.32	4.10±0.31	2.18±0.24***
Urinary Volume (ml)	0	203±12.13	180±9.92	200±16.28	211±15.31
	11	200±9.16	168±11.96	161.33±9.70	161.33±17.55
	21	217±19.77**	126±9.09	148±18.21	136±17.86

Results were expressed as Mean ± Standard error of the mean * sign shows significantly different from gentamicin treated group, ** very significant and *** extremely significant

CONCLUSION

It can be concluded from the present study that despite strong flavonoids and antioxidant properties, *V. wallichii* failed to produce significant nephroprotective results assessed by various physiological, biochemical and histological parameters.

ACKNOWLEDGEMENT

Higher Education Commission Islamabad is highly acknowledged for their financial support for conducting the project through indigenous 5000 scholarships.

REFERENCES

Baquar SR (1989). Medicinal and poisonous plants of Pakistan, Karachi, Printas, pp.479-480.

Bate-Smith EC (1962). The Phenolic constituents of plants and their taxonomic significance, Dicotyledons. *J. Linn. Soc. Bot*., **58**: 95-173.

Bennett WM, Elzinga LW and Porter GA (1991). Tubulointerstitial disease and Toxic nephropathy. In: Brenner BM, Rector FC editors. The Kidney, 4th ed., WB Saunders Co., Philadelphia, pp.1430-1496.

Blosser N (1985). Electrolytes. *In*: Clinical Chemistry; Principles, procedures, correlations. Bishop ML,

Duben-Von Laufen JL, Fody EP editors. Lippincott., Philadelphia, pp.263-289.

Brinker KR, Bulger RE and Dobyan DC (1981). Effect of potassium depletion on gentamicin nephrotoxicity. *J. lab. Clin. Med*., **99**: 156-162.

Chow JW, Vine MJ, Shlaes DM, Quinn JP, Hooper DC, Johnson MP, Ramphal R, Wagener MM, Miyashiro DK and Yu VL (1991). Enterobacter bacteremia; clinical features and emergence of antibiotic resistance during therapy. *Ann. Intern. Med.*, **115**: 585-590.

Cronin RE, Bulger RE, Southern P and Henrich WL (1980). Natural history of aminoglycoside nephrotoxicity in the dog. *J. Lab. Clin. Med*., **95**: 463-474.

Cronin RE, Nix KL, Ferguson ER, Southern PM and Henrich WL (1982). Renal cortex ion composition and Na^+-K^+-ATPase activity in gentamicin nephrotoxicity. *Am. J. Physiol*., **242**: 477-483.

Deutsche GKC (1972). Standardisierung von Methoden zur Bestimmung von Enzymaktivitaten in biologischen flussigkeiten (Recommendation of the German Society of Clinical Chemistry. Standardization of methods for measurement of enzymatic activities in biological fluids). *Z. klin. Chem. Klin. Biochem*., **10**: 182-192.

Furuta N and Nakada J (1993). Study on gamma-GTP activity in urine and renal tissue of drug induced nephrotoxicity in rats. *Nippon Hinyokika Gakkai Zasshi*, **84**(7): 1197-1205.

Gilani AH, Khan AU, Jabeen Q, Subhan F and Ghafar R (2005). Antispasmodic and blood pressure lowering effects of *Valeriana wallichii* are mediated through K+ channel activation. *J. Ethnopharmacol.,* **100**(3): 347-52.

Gilbert DN, Wood CA, Kohlhepp SJ, Kohnen PW, Houghton DC, Finkbeiner HC, Lindsley J and Bennett WM (1989). Polyaspartic acid prevents experimental aminoglycoside nephrotoxicity. *J. Infect. Dis*., **159**(5): 945-953.

Gilbert DN and Bennett WM (1993). Polyaspartic acid for the prevention of aminoglycoside nephrotoxicity. Prospectus, 1993.

Jawetz H (1992). Aminoglycosides and Polymyxins. In: Katzung BG editor. Basic and Clinical Pharmacology. 5th ed., Appleton and Lang., pp.645-652.

Johnson AM, Rohlfs EM and Silverman LM (1999). Proteins. In: Burtis CA, Ashwood ER editors. Tietz Text book of Clinical Chemistry. 3rd ed., W.B Saunders Co., Philadelphia, pp.477-540.

Joseph L, Puthallath RE and Rao SN (2015). Acute and chronic toxicity study of *Valeriana wallichii* rhizome hydro-ethanolic extract in Swiss albino mice. *Asian J. Medical Sci.,* **7**(2): 49-54.

Khuda F, Iqbal Z, Zakiullah, Khan A, Nasir F, Muhammad N, Khan JA and Khan MS (2012). Metal analysis, phytotoxic, insecticidal and cytotoxic activities of selected medicinal plants of Khyber pakhtunkhwa. *Pak. J. Pharm. Sci.*, **25**(1): 51-58.

Marder M, Viola H, Wasowski C, Fernandez S, Medina JH and Paladini AC (2003). 6-methylapigenin and hesperidins: New valeriana flavonoids with activity on the CNS. *Pharmacol. Biochem. Behav.*, **75**(3): 537-545.

Niezen JH, Waghorn GC and Charleston WA (1995). Growth and gastrointestinal nematode parasitism in lambs grazing either Lucerne (*Medicago sativa*) or (*Hedysarum coronarium*), which contains condensed tannins. *J. Agri. Sci.*, **125**: 281-289.

Ron BH, Willis CV, Bone K and Morgan M (2000). Herbal products: active constituents, mode of action and quality control. *Nutr. Res. Rev.*, **13**: 47-77.

Smith ST (1985). Non protein nitrogen. In: Bishop ML, Duben-Von Laufen JH, Fody EP editors. In: Clinical chemistry; Principles, procedures, correlations. JB Lippincott Co., Philadelphia, pp. 411-423.

Solez K (1983). Pathogenesis of acute renal failure. *Int. rev. Exp. Pathol*., **24**: 277-333.

Subhan F, Karim N and Ibrar M (2007). Antiinflammatory activity of methanolic and aqueous extracts of *Valeriana wallichii* Dc rizhome. *Pak. J. Pl. Sci*., **13**(2): 103-108.

Suri S and Thind TS (1978). Antibacterial activity of some essential oils. *Indian Drugs and Pharmaceutical Industry*, **13**: 25-28.

Syed SN, Rizvi W, Kumar A, Khan AA, Moin S and Ahsan A (2014). Antioxidant and hepatoprotective activity of ethanol extract of *Valeriana wallichii* in CCl_4 Treated rats. *British J. Pharmaceutical Res*., **4**(8): 1004-1013.

Thompson JR, Simmonsen R, Spindler MA, Southern PM and Cronin RE (1990). Protective effect potassium chloride loading in gentamicin nephrotoxicity. *Am. J. Kidney Dis*., 583-591.

Tripathi SV, Tiwari SK and Chaturveedi GN (1982). Comparative clinical trial of certain indigenous drugs (Dhanya panchaka) in gastric secretion hyperchlorhydria and hyperchlorhydria. *Nagarjun.*, **25**: 170-174.

Tulkens PM (1989). Nephrotoxicity of aminoglycoside antibiotics. *Toxicol. Lett.*, **46**(1-3): 107-123.

Vishwakarma S, Goyal R, Gupta V and Dhar KL (2016). GABAergic effect of valeric acid from *Valeriana wallichii* in amelioration of ICV STZ induced dementia in rats. *Revista Brasileira de Farmacognosia*., **26**(4): 484-489.

Vohra SB and Dandiya PC (1992).Herbal analgesic drugs. *Fitoterapia*., **63** (3): 195-207.

Zager RA (1992). Gentamicin effect on renal ischaemia/ reperfusion injury. *Circ. Res*., **70**(1): 20-28.