Research Article

Nephroprotective effect of *Berberis baluchistanica* against gentamicin–induced nephrotoxicity in rabbit

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Abstract

The present study was conducted to explore the protective effect of *Berberis baluchistanica* against gentamicin-induced renal toxicity in rabbit. Phyto-chemical investigations lead to the isolation of berberine and palmatine. The crude hydro-methanolic extract at various doses (100, 200 and 300 mg/kg body weight) elicited strong nephroprotective effects by restoring various biomarkers which were deranged by gentamicin such as creatinine, urea, serum uric acid levels ($p < 0.001$) in plasma and urine output creatinine clearance, urinary protein and γ-glutamyl transferase level ($p < 0.001$) in urine in dose-dependent manner. The mediators involved in oxidative stress such as malondialdehyde, reduced glutathione, glutathione peroxidase, and catalase levels were significantly ($p < 0.05$-0.001) modulated in kidney tissue homogenate. Correspondingly, there was a significant ($p < 0.05$) recovery in kidney weight and %loss in the body weight compare to gentamicin-treated group. In conclusion, *B. baluchistanica* exhibits nephroprotective effect.

Introduction

Gentamicin is an effective aminoglycoside antibiotic used against infections caused by Gram positive and Gram negative aerobic bacteria. But nephrotoxicity and oxidative damage limit its long-term clinical use (Alarifi et al., 2012).

The oxidative stress are diminished upon supple-mentation with certain dietary anti-oxidants such as vitamin E, C, these vitamins was seems to ameliorate the suppression in enzymatic activity of GSH-Px and catalase in the renal tissues induced by gentamicin (Kavutcu et al., 1996). The other non-nutrient anti-oxidant such as flavonoids, medicines derived from plant extracts are being increasingly utilized as adjunct treatment options for a wide variety of clinical disease (Rahimi et al., 2005; Al-Azzawie and Alhamdani, 2006).

More interest has developed to the protective effects of medicinal plants against chemically induced toxicities (Frei and Higdon, 2003). For example, *Azima tetracantha* (Konda et al., 2016) and *Capparis spinosa* (Tlili et al., 2017), *Cestrum nocturnum* (Saleem et al., 2017), *Morus alba* (Ullah et al., 2016), *Nigella sativa* (Begum et al., 2006), Sechium edule (Mumtaz et al., 2012), *Terminalia muelleri (*Fahmy et al., 2016) show nephroprotective effect.

Berberis baluchistanica (Family *Berberidaceae*), which in local language "Brahvi" known as Zarch, found in Kalat district, Balochistan province, Pakistan. It is a wild plant and is used as medicine by the local people, roots decoction is used for cure of internal injuries. Beside this, it is also used for the removal of kidney related disorders as it contains berberine which is known to be useful in the treatment of number of diseases (Baloch et

al., 2013). Strong antibacterial effects of the roots extracts against a variety of pathogenic bacteria have been reported (Kakar et al., 2012). The phytochemical investigations showed that the genus, berberis is rich in berberine and berberine like compounds, while from *B. baluchistanica* several alkaloids such as pakistanine, pakistanamine and (+)-baluchistine have been isolated (Shamma et al., 1973; Miana et al., 1979).

Keeping in mind, the strong *in vitro* anti-oxidant potential and traditional use of *B. baluchistanica* in the treatment of kidney disorders, the present study was designed to evaluate the nephroprotective like effect of *B. baluchistanica* against gentamicin-induced nephrotoxicity.

Materials and Methods

Chemicals

Gentamicin sulfate (80 mg/2 mL; Abbott Laboratories Pakistan Ltd.), silliver® (Silymarin, 200 mg; Searl Pakistan Ltd.), reduced glutathione, glutathione reductase, thiobarbituric acid, fosfotungustic acid, hydrogen peroxide, NADPH and other reagents were used.

Plant material

The roots of the plant (35 kg dry wt.) of *B. baluchistanica* were collected from, District Kalat, Balochistan Province, Pakistan, during the month of April 2012. The plant was identified by Prof. Muhmmad Ibrar of the Department of Botany, University of Peshawar. A voucher No. Bot/20105 has been placed in the herbarium of the Department of Botany, University of Peshawar as a reference.

Preparation of plant extracts

The freshly collected shade dried roots was powdered and extracted by maceration in 90% methanol for 10 days (3 × 50 L). The combined methanol extract was filtered with a muslin cloth, evaporated and concentrated under vacuum using rotary evaporator (Buchi – R210, Switzerland) at a temperature of 40°C and a viscous extract (2.2 kg) was obtained. The crude methanolic extract was investigated for the nephroprotective activity.

Phytochemical investigation

The *B. baluchistanica* extract was screened for alkaloids, flavonoids, saponins, phenols, and diterpines (Baloch et al., 2013) The crude extract is further screened for the presence of triterpine, tannins and saponins.

Isolation and structure elucidation of compounds

The viscous extract (2.2 kg) was mixed with 3L distilled water and extracted three times with 6L *n*-hexane, chloroform, ethyl-acetate, and *n*-butanol to get *n*-hexane

(59 g), ethyl acetate (39 g), chloroform (88 g), butanol (65 g) and water (89 g) soluble fractions respectively. The ethyl acetate soluble subfraction was chromatographed over silica gel and eluted with mixtures of *n*-hexane-chloroform, and chloroform–methanol in increasing order of polarity to obtain 8 major fractions EtA$_1$- EtA$_8$. Fraction EA$_3$ obtained from *n*-hexane–chloroform (2.0:8.0) was rechromatographed and eluted with chloroform–methanol (4.5:5.5 and 2.0:8.0) to afford compound (**1**) (12 mg), and compound (**2**) (14 mg), respectively. The purity of compounds were analyzed using TLC followed by spraying with Dragendorff's reagent. Structures were elucidated using various techniques such as ^{1}H-NMR, ^{13}C-NMR, 2D-NMR, EI-MS, FAB-MS, UV and IR.

Animals and experimental layout

The healthy male rabbits (*Oryctolagus cuniculus*) (1,200-1,500 g) were purchased from the Veterinary Research Institute, Peshawar and maintained under optimal conditions at the University of Peshawar. The rabbits were fed on chow pellets along with fresh green vegetables and grasses and free access to fresh water *ad libitum*. Before feeding experimental diet, animals were acclimatized for two weeks.

Animals grouping and dosing

The rabbits were divided into groups, each having six animals. Three doses (100, 200 and 300 mg/kg p.o) were tested for the crude extract. During the experiment, gentamicin 100 mg/kg i.p. while silymarin at 50 mg/kg i.p. were administered (Shahbazi et al., 2012).

Group 1: Normal saline (control); Group 2: gentamicin (100 mg/kg/day, i.p); Group 3: silymarin (50 mg/kg/day, i.p); Groups 4: methanol extract (100 mg/kg/day, p.o) plus gentamicin (100 mg/kg/day, i.p); Groups 5: methanol extract (200 mg/kg/day, p.o) plus gentamicin (100 mg/kg/day, i.p); Groups 6: methanol extract (300 mg/kg/day, p.o) plus gentamicin (100 mg/kg/day, i.p). The experiment was continue for 15 days

Samples collection and processing

After dosage completion, 24 hours later on day 16, rabbits were anesthetized by intraperitoneal injection of ketamine. The blood samples were taken by cardiac puncture, transferred to EDTA and non-EDTA containing tubes. The serum was separated by centrifugation (K240R, Centurion Scientific, UK) for 15 min at 3,000 rpm and 37°C, stored at 4°C till determination of biochemical parameters. The serum sample was assayed for urea, creatinine, and uric acid through standard procedures (Cobas c 311 analyzer kit, UK).

Collection and analysis of urine

At the end of the experimental period, 24 hours urine

samples were collected in standard metabolic cages from each animal in all the groups. After measuring volume, these urine samples were used for analyze for urinary protein, creatinine clearance and gamma-glutamyl transferase levels. These parameters were estimated through COBAS chemistry automation using Roche Diagnostic kits.

Measurement of body and organs weight

The body weight was measured on a daily basis throughout the treatment period before administration of drug or saline and at the end of experiment in all the groups. Similarly, at the end of 15-day treatment, each rabbit was euthanized and theabdominal cavity was dissected out and liver was removed and weighted.

Preparation of liver homogenate

The right kidney was immediately removed, washed thoroughly in the cold saline solution to remove the blood. The 10% homogenate was prepared in phosphate buffer (0.05M, pH 7) using a homogenizer at 25° C. The homogenate was centrifuged to remove the cell debris and the supernatant was used for the determination of *in vivo* anti-oxidant assay.

In vivo anti-oxidant activities

The levels of lipid peroxidation is based on the reaction of malondialdehyde, a product of lipid peroxidation with thiobarbituric acid to form thiobarbituric acid reactive substances, which has absorption at 514 nm and shows pink color (Placer et al., 1966; Rao and Mohan, 2017).

Catalase activity was determined according to the method of Aebi (1984). In brief, kidney tissue homogenate supernatant approximately 10.5 mL was added to 3 mL of phosphate-buffered saline and the absorbance was read at 240 nm using a UV spectrophotometer. The principle of the assay is based on the H_2O_2 decomposition rate at 240 nm. Results were obtained kg/ protein.

Glutathione peroxidase (GSH-Px) was measured by the method of Goldberg and Spooner described as oxidized glutathione is instantly converted to its reduced form with a simultaneous oxidation of NADPH to NADP+ (Burk et al., 1980). In the presence of glutathione reductase and NADPH, The decrease in absorbance at 340 nm is measured. GSH-Px activity was expressed as U/mg protein. Tissue reduced glutathione activity was determined according to the previously published method (Kaur et al., 2006). The kidney tissue homogenate 0.8 mL was mixed with 0.5 mL 20% solution of trichloroacetic acid, the mixture was kept on ice bath for 10 min, then subjected to centrifugation at 3,000 rpm for 15 min, 0.5 mL of the supernatant was mixed 2 mL of 0.6 mM dithionitrobenzene (prepared in 0.2M buffer, pH 8) with 0.8 mL of 0.2M sodium phosphate buffer (pH 8) when yellow color obtained absorbance was measured at after 20 min at 412 nm The reduced glutathione content was expressed as U/mg protein.

Histopathalogical examination

For histopathalogical examination, the left kidney was fixed in 10% buffered formalin for 48 hours and was dehydrated in graded ethanol solution and embedded in paraffin blocks. The sections of the kidney were oriented perpendicular to the plane of section in the block and 6 micrometer thick transverse sections were cut and mounted on the glass slides and stained with Hematoxylin and Eosin (Tomita et al., 1993; Miyamoto

Table I		
Phytochemical screening of *B. baluchistanica*		
Test	Observation	Result
1 drop of extract on TLC plate + Dragendorff's reagent	Appearance of orange /red color	Alkaloids present
150 mg extract + 1.5 mL chloroform → warmed for 0.5 hour → 1 mL sulphuric acid added	No color change observed	Tri-terpenoids absent
100 mg extact → boil + 2.5 mL distilled water → shake→ foam formation	No foam appear	Saponin absent
250 mg extract + 2.5 mL dilute ammonia solution → 0.5 mL sulphuric acid added	Appearance of yellowish color	Flavonoids present
Aqueous aliquot of extract + ferric chloride reagent	Appearance of greenish black color	Tannins present
150 mg extract + sulphuric acid → boil/cool→ add chloroform → separate chloroform layer + dilute ammonia solution	No color change	Anthraquinone absent
1 g extract + 10 mL of distilled water + add few drops of copper acetate→	Emerald green color	Diterpenes present
500 mg of MBHE + 0.5 mL Fehling A solution + 0.5 mL Fehling solutions + heat	Not any change observed	Reducing sugar absent

Table II						
Effect of _B. baluchistanica_ extract on gentamicin-induced nephrotoxic rabbit						
Groups	Normal saline (10 mL/kg)	Gentamicin (80 mg/kg)	Silymarin (50 mg/kg)	Methanol extract (100 mg/kg) plus gentamicin	Methanol extract (200 mg/kg) plus gentamicin	Methanol extract (300 mg/kg) plus gentamicin
Kidney weight (g/100 g bw)	0.8 ± 0.1	1.1 ± 0.1	0.9 ± 0.1[c]	1.0 ± 0.1[b]	0.8 ± 0.1[b]	0.8 ± 0.1[c]
%Loss in body weight	0.2 ± 0.9	10.8 ± 1.1[a]	2.7 ± 0.9[c]	3.6 ± 0.8[c]	4.7 ± 0.9[c]	5.6 ± 1.5[c]
Blood urea (mg/dL)	12.0 ± 2.6	24.3 ± 2.3[a]	11.5 ± 3.4[b]	14.5 ± 1.5[c]	12.3 ± 1.3[b]	10.3 ± 2.0[c]
Blood uric acid (mg/dL)	2.5 ± 0.0	4.2 ± 0.2[a]	1.2 ± 0.2[b]	2.9 ± 0.2[b]	1.6 ± 0.3[a]	1.1 ± 0.2[c]
Serum creatinine (mg/dL)	0.5 ± 0.1	3.5 ± 0.3[a]	0.4 ± 0.3[b]	0.5 ± 0.1[c]	0.4 ± 0.0[b]	0.2 ± .1[c]
Urine output (mL/hour)	200 ± 9.2	168 ± 12.0[a]	200.6 ± 19.6[b]	199 ± 26.0[b]	200 ± 26.0[b]	208 ± 21.8[c]
Creatinine clearance (mL/min)	4.7 ± 2.8	0.4 ± 1.3[a]	1.7 ± 0.8	3.3 ± 1.0[c]	4.0 ± 0.9[b]	4.7 ± 1.0[c]
Urinary protein (mg/dL)	1.8 ± 0.2	3.86 ± 0.3[a]	1.74 ± 0.1[b]	1.7 ± 0.3[c]	1.8 ± 0.2[b]	1.7 ± 0.3[c]
Urinary γ-glutamyl transferase (U/L)	48.8 ± 5.1	104.7 ± 8.1[a]	50.6 ± 4.2[b]	72.7 ± 3.2[b]	60.6 ± 2.2[c]	56.4 ± 1.1[c]
MDA (nmolg/protein)	4.8 ± 0.6	12.3 ± 1.9[a]	4.9 ± 0.7	10.5 ± 1.0[b]	6.1 ± 0.3[c]	5.2 ± 0.3[c]
Catalase (kg/protein)	133.0 ± 11.4	70.3 ± 1.9[a]	137.0 ± 11.5[c]	90.4 ± 2.2[b]	103.5 ± 7.3[b]	121.5 ± 8.3[c]
GSH (nmol g/tissue)	2.0 ± 0.0	1.8 ± 0.0	2.2 ± 0.4[a]	1.8 ± 0.0[a]	2.0 ± 0.3[b]	2.3 ± 0.2[b]
GSH-Px (IU/g protein)	33.0 ± 1.2	18.7 ± 3.0[a]	33.9 ± 1.5[b]	24.3 ± 2.0[b]	26.4 ± 1.6[b]	27.3 ± 2.4[b]
Histopathological findings						
Interstitial inflammation		++		-	-	-
Tubular dilatation		+++		+	+	+
Necrosis of epithelium		++		-	-	-
Glomerular congestion		++		-	-	-
Tubular cast		++		+	-	-
Data are expressed as mean ± SEM followed by Tukey's _post hoc_ test;. [a]p<0.05 significant difference compared to the normal control; [b]p<0.001, [c]p significant difference compare to gentamicin-treated group (n = 6); (–) none; (+) mild; (++) moderate; (+++) severe						

et al., 2002). The microscopic observation (Labomed Lx400 with digital camera iVu 3100, USA) was done to keep the count of histopathological changes, which were graded as (–) none; (+) mild; (++) moderate; (+++) severe (Shahid and Subhan, 2014; Shahid et al., 2016).

Statistical analysis

Data are presented as means ± (SEM). To compare means, all groups were subjected to one-way analysis of variance (ANOVA) followed by Tukey' smultiple comparison post hoc test using GraphPad Prism5 (GraphPad Software, USA), to determine the difference in all parameters. Results were considered statistically significant at p<0.05.

Results

Preliminary phytochemical analysis

Phytochemical investigation of the methanol extract revealed the presence of alkaloids, flavonoids and tanins (Table I).

Isolated compounds

Compound **1** (berberine; Figure 1) was isolated from the ethyl acetate fraction through normal phase column chromatography using normal phase silica gel as stationary phase. Mobile phase used was CHCl$_3$–MeOH (4.5:5.5). The EI-MS showed a molecular ion peak at 336.1295. High resolution EI-MS confirmed the molecular formula to be C$_{20}$H$_{18}$NO$_4$.

The broad band ^{13}C-NMR showed 20 signals, out of which six were methines and three methylenes confirmed by dept 90° and dept 135° respectively.

H-1 resonated at δ 7.656 showing HMBC correlation with C-2 (δ 149.918) and C-3 (δ 152.174). H-4 resonated at δ 6.957 (s) showing HMBC correlation with C-3 (δ 152.174), C-2 (δ 149.918) and C-14a (δ121.855). The methylene protons H-5 resonated at δ 3.261 as triplet and H-6 at δ 4.930 as triplet. H-5 showed HMBC correlation with C-4a (δ131.884), C-4 (δ109.397), C-6

(δ57.203) and C-14a (δ121.855). The correlation of H-5 and H-6 was also confirmed by COSY spectrum. H-6 methyle protons showed HMBC correlation with C-4a (δ 131.884), C-5 (δ 28.20), C-8 (δ 146.403) and C-14 (δ 139.661). H-8 proton resonated at δ 9.764 as singlet, showed HMBC correlation with C-8a (δ 123.321), C-9 (δ 145.770) and C-14 (δ 139.661). H-11 resonated as doublet at δ 8.1211, showed HMBC interaction with C-9 (δ 149.770), C-10 (δ 152.022) and C-12 (δ 124.521). H-12 methine protons resonated at δ 8.005 as doublet shoed HMBC correlation with C-9 (δ 145.770), C-10 (δ 152.022), C-12a (δ 135.162) and C-13 (δ 121.482). the COSY spectrum showed the correlation of H-11 and H-12.

H-13 showed resonance signals as singlet at δ8.70. the HMBC interrelation of H-13with C-8a (δ 123.321) and C-14 (δ 139.661) was observed. The methylene protons of O-CH₂-O group resonated as singlet at δ 6.105. The HMBC spectrum showed correlation of this methylene with C-2 (δ 149.918) and C-3 (δ 152.174).

Two methyls resonated as singlets at δ 4.200 and δ 4.104 in H-NMR spectrum. These singlets were assigned to two methoxy groups attached with C-9 (δ 145.770) and C-10 (δ 152.022) confirmed by HMBC spectrum.

Compound **2** (palmatine; Figure 1) was isolated from the ethyl acetate fraction using repeated column chromatography The EI-MS shows the molecular ion peak at m/z: 352 [M+] (calculated for $C_{21}H_{22}NO_4^+$). The HREI-MS was in agreement with molecular formula for $C_{21}H_{22}NO_4^+$) at m/z (rel. int. %): 352 (11, M+), 351 (35), 337 (100), 294 (46), 278 (34), 219 (7), 190 (8), 138 (5), 94 (5), 43 (10).

1HNMR of compound **2** showed six aromatic protons at δ 7.83, (1H, s, H-1), 7.23 (1H, s, H-4), 9.75 (1H, s, H-8), 8.40 (1H, d, J = 9.0 Hz, H-11), 8.05 (1H, d, J = 9.0 Hz, H-12) and 9.12 (1H, s, H-13), three methylenes at δ 6.09 (2H, s, H-14), 3.24 (2H, t, J = 10.0 Hz, H-5) and 4.23 (2H, s, J = 10.0 Hz, H-6) and two methoxy peaks at δ 4.06 (3H, s, 9-OMe) and 4.09 (3H, s, 10-OMe).

The ¹³C-NMR spectrum of compound **2** showed twenty one carbon atoms which included six methine, two methylene, four methoxy and nine quaternary carbon atoms on the basis of DEPT-q spectral data.

The methine proton at δ 9.12 (H-8) showed correlations with δ 138.75 (C-13a), 56.43 (C-6), 123.33 (C-8a), 135.10 (C-12a) and 145.40 (C-9), the methine proton at δ 8.40 (H-11) correlated to δ 148.68 (C-10), 124.30 (C-12), 135.10 (C-12a) and 145.40 (C-9), the methine proton appearing at δ 8.04 (H-12) showed correlations with δ 125.53 (C-11), 148.68 (C-10), 135.10 (C-12a), 123.33 (C-8a) and 118.81 (C-13), the methine proton at δ 9.12 (H-13) correlated to δ 135.10 (C-12a), 123.33 (C-8a), 138.75 (C-13a) and 123.40 (C-12).

The methylene protons appearing at δ 3.23 (H-5)

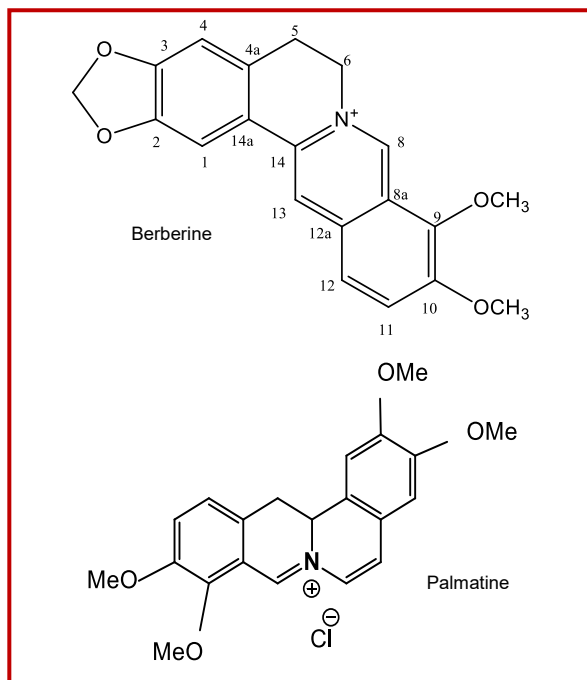

Figure 1: Chemical structure of berberine and palmatine

correlated to δ 56.43 (C-6), 127.45 (C-4a), 119.80 (C-13b) and 113.25 (C-4), the methylene protons at δ 4.84 (H-6) showed correlations with δ 138.75 (C-13a), 143.35 (C-8), 127.45 (C-4a) and 24.84 (C-5) and four methoxy peaks at δ 4.09 (9-OMe), 4.06 (10-OMe), 3.24 (2-OMe) and 4.23 (3-OMe) correlated to δ 145.40 (C-9) , 148.68 (C-10), 152.52 (C-2) and 152.23 (C-3) respectively.

The data of the compounds were clearly matched with reported data from the literature of berberine and palmatine (Küpeli et al., 2002).

Effect on physical parameters

The treatment with the extract significantly (p<0.05) improved the weight changes induced by gentamicin. The overall effect was dose-dependent at 100, 200 and 300 mg/kg p.o. Similarly, the methanol extract caused significant (p<0.05) recovery in the kidney weight and %loss in the body weight compare to gentamicin-treated group.

Effect on biochemical markers

The treatment of roots extract of the plant significantly (p<0.05) ameliorated the toxicological changes induced by gentamicin in blood urea, serum uric acid and creatinine levels. The overall effect was dose-dependent at 100, 200 and 300 mg/kg p.o. Similarly, the crude extract of the plant caused significant (p<0.05) nephro-protective effect on the urine biomarker including crea-tinine clearance, gamma-glutamyl transferase, urinary output and urinary protein levels.

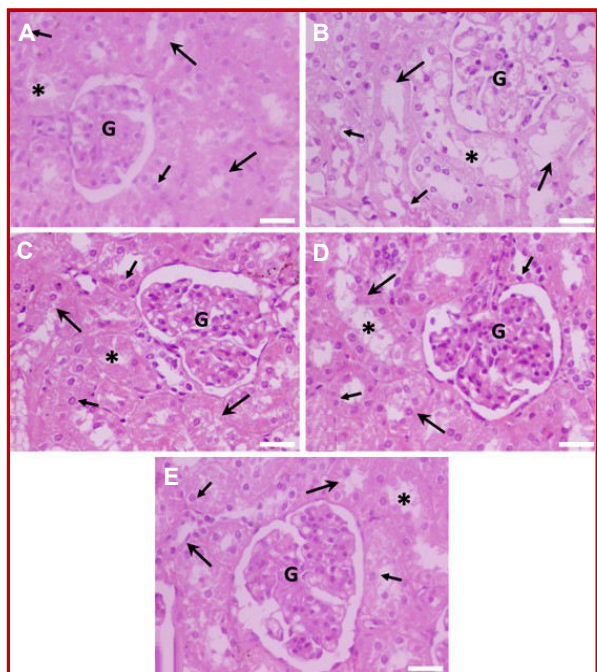### Effect on oxidative stress biomarkers

Figure 2: Histopathological evaluation of *B. baluchistanica* on GM-induced nephrotoxicity (H and E staining; x40, scale bar =100 µm) (n = 6 rabbits per group). (A): Photomicrograph of a section of kidneys from a rabbit treated with normal saline/vehicle showing normal appearance of glomerulus (G) in a Bowman's capsule interspersed among renal tubules (large arrows) lined by epithelial cells (small arrows) with a brush border extending into the lumen (asterisk). (B): Photomicrograph of a section of kidney from a rabbit treated with Gentamicin (100 mg/kg) showing mild atrophy of glomerulus (G), inflammation of interstitial spaces, dilatation of renal tubules (large arrows) containing cellular cast (small arrows) in their lumen (asterisk). Normal histological appearance of glomerulus (G), renal tubules (large arrows) lined by epithelial cells (small arrows) with a brush border extending into the lumen (asterisk) are observed in groups of rabbits treated with (C): mBBE (100 mg/kg) plus GM, (D): mBBE (200 mg/kg) plus GM, and (E): mBBE (300 mg/kg) plus GM.

Animals treated with gentamicin showed significant increase in kidney malondialdehyde, while animals treated along with crude methanolic extract (all doses) significantly controlled the increase in kidney malondialdehyde level. Similarly catalase, reduced glutathione and GSH-PX activity were decreased in gentamicin-treated group while groups treated with crude methanol extract significantly recovered the levels of above biomarkers.

Effects of gentamicin and B. baluchistanica on kidney histopathology

Histological findings of this study showed photomicrograph of a section of kidney from a rabbit treated with normal saline/vehicle showing normal appearance of glomerulus in a Bowman's capsule interspersed among renal tubules lined by epithelial cells with a brush border extending into the lumen

(Figure 2A). Similarly, Figure 2B demonstrated the section of kidney from a rabbit treated with gentamicin (100 mg/kg) representing mild atrophy of glomerulus, inflammation of interstitial spaces, dilatation of renal tubules, containing cellular cast in their lumen. Correspondingly improved architecture is observed in crude methanolic extract-treated groups (100 mg/kg) plus gentamicin, (Figure 2C), crude methanolic extract (200 mg/kg) plus gentamicin, (Figure 2D) and crude methanolic extract (300 mg/kg) plus gentamicin (Figure 2E) in dose-dependent manner with marked histological amelioration compared with the gentamicin-treated group.

Discussion

The current study revealed significant nephroprotective like effect of crude methanol extract *B. baluchistanica* roots in gentamicin-induced toxicity by ameliorating biochemical markers and physical parameters strongly augment by histopathalogical changes in experimental rabbits.

The exact mechanism by which gentamicin induced nephrotoxicity is unknown, however, several researchers reported that aminoglycoside antibiotics are a class of drug capable of causing the formation of reactive oxidative species (ROS) which can be directly involved in gentamicin-induced damage. Malondialdehyde, end product of lipid peroxidation in tissues, results in a decrease in polyunsaturated fatty acid content, which serves as substrate for free radicals (Eisenberg et al., 1987). It is also reported that iron is important in models of tissue injury. Gentamicin acts as an iron chelator and that have been shown to cause release of iron from renal cortical mitochondria and iron-gentamicin complex is a potent catalyst of free-radical formation and enhance the generation of ROS (Priuska and Schacht, 1995; Baliga et al., 1999). GGT levels may be a prominent and sensitive marker of oxidative stress (Lee et al., 2004). Studies have shown that GGT, like other anti-oxidant enzymes, is inducible by oxidative stress, which increases GGT activity (Zhang et al., 2006). The impairment in kidney functions was accompanied by either increase in serum creatinine and urea levels or kidney tissue malondialdehyde levels that indicated lipid peroxidation (Cuzzocrea et al., 2002). The reduced glutathione is one of the essential compounds for maintaining cell integrity participation in the cell metabolism. However, xenobiotics or peroxide-dependent alterations in tissue reduced glutathione levels and anti-oxidant enzyme activities are currently controversial problem. In another study (Ateşşahin et al. 2003), it was found that gentamicin caused depletion in kidney reduced glutathione levels and unaffected anti-oxidant enzyme activities such as GSH-Px and catalase. Controversial

results are reported by several investigations (Nakajima et al., 1994; Maldonado et al., 2003; Parlakpinar et al., 2005). Gentamicin-induced nephrotoxicity was associated with low activity of GSH-Px, catalase, and levels of reduced glutathione in the renal cortex. These decreases in renal anti-oxidant enzymatic protection could aggravate the oxidative damage. The increased production of ROS in gentamicin-induced nephrotoxicity may cause inactivation of anti-oxidant enzymes such as GSH-Px, catalase. Similarly, in this study, the increases in malondialdehyde levels, decreases in GSH-Px and catalase activities, but no alteration in reduced glutathione levels in rabbits treated with gentamicin alone appear to support above investigations that gentamicin-induced nephrotoxicity is associated with lipid peroxidation in renal tissue as reflected by an increase in malondialdehyde. These observations are correlated well with the renal histological findings, which explained extensive and marked tubular necrosis. The nephroprotection by medicinal plants may be by inhibiting the production of ROS and lipid peroxidation (Fahmi et al., 2016; Konda et al., 2016; Tilili et al., 2017).

B. baluchistanica is a wild plant used as medicine by local people. They used the roots decoction for the cure of internal injuries and ophthalmic problems. Beside of this, it is also used for the removal of kidney stones, as it contains berberine and berberine like compounds, which is known to be useful for a number of diseases (Li et al., 2014).

A marked restoration in lipid peroxidation paralleled with GSH-Px and catalase activity in methanol extract-treated group were observed. These findings were in conformity with above parameters, as similar results observed in this study. Phytochemical strength of genus Berberis is berberine, an isoquinolone alkaloid found in many plants of genus berberis and its derivatives (Imanshahidi and Hosseinzadeh, 2008; Guamán Ortiz et al., 2015; Khan, 2016). These compounds possessed strong *in vitro* and *in vivo* anti-oxidant potentials (Somova et al., 2003; Lyamzaev et al., 2011) which support nephroprotective like effect of *B. baluchistanica*. Interestingly, it is reported that berberine at the doses of 3 mg/kg caused significant neprohroprotective like effect (Kumar et al., 2015). In another study, berberine ameliorates proteinuria in type 2 diabetic (Liu et al., 2008; Ashraf et al., 2013). It is also reported that berberine reduce oxidative stress in streptozotocin diabetic rats (Moghaddam et al., 2014). In this phytochemical investigation, it had been isolated a number of compounds including berberine and palmatine. Therefore, the current findings on methanol extract can be attributed to these isolated compounds.

Conclusion

B. baluchistanica root extract possesses marked

protective effect against gentamicin-induced nephrotoxicity by ameliorating various biomarkers of nephrotoxicity and oxidative stress.

Ethical Issue

The study protocols were approved by the Ethical Committee of Department of Pharmacy, University of Peshawar (01/EC-17/pharm).

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