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Barleria prionitis L. extracts ameliorate doxorubicin-induced acute kidney injury via modulation of oxidative stress, inflammation, and apoptosis

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Background and aim: Doxorubicin (DOX) is a chemotherapeutic drug with potential nephrotoxic effects on patients who are on cancer chemotherapy. An interest has been observed in using natural products to ameliorate the potential side effects of DOX. The present study is to investigate the cellular mechanisms underlying the protective effects of *Barleria prionitis* L. (BP) (Acanthaceae) extracts, DOX-induced acute kidney injury (AKI).

Experimental procedure: Hexane (25 mg/kg/day), ethyl acetate (80 mg/kg/day), n-butanol (70 mg/kg/day), and water (120 mg/kg/day) extracts of BP, were administered to DOX-induced (5 mg/kg (2500 μL/kg), ip) Wistar rats for four consecutive weeks. At the end of the study, investigations were carried out for the assessment of biomarkers of nephrotoxicity, oxidative stress, inflammation, and apoptosis.

Results: Treatments with BP extracts significantly reversed DOX-induced elevations in serum and urine biochemical markers of nephrotoxicity (serum creatinine; 21–33%, blood urea nitrogen; 26–58%, β_2-microglobulin; 19–22% and urine total protein; 47–67%). There was a reduction in the levels of tumor necrosis factor-α, interleukin-1β, and malondialdehyde in kidney homogenates of rats treated with the n-butanol extract (by 43, 62, and 24%) and water extract (by 57%, 85%, and 26%) ($p < 0.05$). Immunohistochemical expression of the pro-apoptotic B-cell associated X protein was reduced while the anti-apoptotic B-cell lymphoma gene product 2 protein was increased in kidney tissues after the treatments with BP extracts.

Conclusions: The selected BP extracts significantly ameliorated DOX-induced AKI. The findings would open new vistas for the development of a drug using the BP extracts to minimize DOX-induced AKI in cancer patients.

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1. Introduction

Drug-induced acute kidney injury (AKI) has become a severe health problem worldwide due to the wide use of pharmaceutical agents with potential toxicities.[1] The high blood perfusion and the functions of kidneys in drug metabolism make them highly susceptible to the direct toxic effects of xenobiotics.[2,3] However, the clinical manifestations of drug-induced AKI are often unrecognized, particularly in a short duration of drug exposure. This may cause issues in the precise assessment of the incidence, severity, and long-term consequences of kidney damage.[4] AKI extends to the late diagnosis of kidney failure, particularly after the manifestation of end-stage renal disease leading to significant mortality.[5] Furthermore, the survivors of AKI appeared to be predisposed to chronic

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List of abbreviations

AKI	acute kidney injury
BCL-2	B-cell lymphoma gene product 2
Bax	B-cell associated X protein
BP	*Barleria prionitis*
BPEA	*Barleria prionitis* ethyl acetate extract
BPH	*Barleria prionitis* hexane extract
BPNB	*Barleria prionitis* n-butanol extract
BPW	*Barleria prionitis* water extract
COX-2	cyclooxygenase 2
DOX	doxorubicin
DPPH	1, 1′-diphenyl-2-picrylhydrazyl
FS	fosinopril sodium
GAE	gallic acid equivalents
GC-MS	gas chromatography-mass spectrometry
IL-1β	interleukin-1β
NC	normal control
QE	quercetin equivalents
TLC	thin-layer chromatography
TNF-α	tumor necrosis factor-α

kidney disease due to maladaptive repair mechanisms following AKI. A study revealed that about 50% of the patients who survived AKI, had residual structural and functional defects which might lead to subsequent episodes of kidney failure or a state of end-stage renal disease.[6]

Several potent therapeutic drugs have been found to exert nephrotoxic effects through different pathogenic mechanisms in different segments of the nephron.[4,7] However, studies have shown that the nephrotoxicity of chemotherapeutic drugs remains a significant drawback to the successful treatment of cancers. Approximately 50–60% of patients who undergo cancer chemotherapy develop AKI leading to increased morbidity and mortality.[8] Doxorubicin (DOX) is one such chemotherapeutic drug with potential nephrotoxic effects that can cause temporary or permanent kidney injury in patients who are on cancer chemotherapy.[9,10] The nephrotoxic effect of DOX is attributed to the decline in oxidative, inflammatory, and apoptotic renal profiles in several scientific reports.[11,12] In recent decades, cancer biologists have shown great interest in the clinical use of natural products to complement the therapeutic and potential side effects of cancer chemotherapy.[13,14] Natural products, particularly medicinal herbs, possess superior structural and chemical diversity compared to synthetic libraries, making them the best-known sources of novel drugs and drug leads.[15] *Barleria prionitis* L. (BP) (Acanthaceae), generally known as “Porcupine flower”, is one of the most commonly used medicinal herbs in conventional medicine with scientifically proven anticancer effects. Its pharmacological significance includes antidiabetic, antioxidant, anti-inflammatory, anticataract, antihypertensive, antiarthritic, antinociceptive, antibacterial, antifungal, hepatoprotective, analgesic, antihelminthic effects, etc.[16,17] Phytoconstituents such as iridoid diglucosides, secoiridoids, terpenoids, triterpenes, phenolic acid, flavones, and phytosterols were isolated from the plant. The antioxidant, anti-inflammatory, and free radical scavenging properties were reported for the isolated phytoconstituents.[16] More importantly lupeol, vanillic acid, syringic acid, 6-hydroxyflavone, β-sitosterol, and p-hydroxybenzoic acid exerted potential anticancer effects.[16] The pharmacological significance in cancer treatment is further upgraded with the synthesis of platinum and palladium nanoparticles from BP.[18]

The plant is among the common remedies used in the management of kidney-related diseases in traditional systems of medicine.[19] however, studies on investigation of potential nephroprotective principles are quite limited. We previously reported the efficacy of the standardized aqueous extract of the whole plant of BP in a nephrotoxicity animal model.[20] Yet, the exact mechanisms of nephroprotection by the plant have not been investigated in that particular study. Investigation of possible protective mechanisms by which the plant reduces nephrotoxicity is an important consideration in the process of developing potential therapeutics/adjuvants to assist conventional cancer chemotherapy.[14] Therefore, herein we investigated the possible cellular mechanisms underlying the protective effects of selected extracts of BP on DOX-induced AKI.

2. Materials and methods

2.1. Chemicals and reagents

All general reagents and chemicals, used in the study were analytical grade and obtained from Sigma-Aldrich (USA).

2.2. Plant material and authentication

The whole plant materials of BP were collected in the flowering stage, in March 2019, from Galle, Sri Lanka. The plant materials were taxonomically identified and authenticated by Dr. N.P.T. Gunawardena, Taxonomist at the National Herbarium, Sri Lanka. An herbarium specimen (PG/2016/55/03) was deposited at the Mini Herbarium, Department of Biochemistry, Faculty of Medicine, University of Ruhuna.

2.3. Plant extraction

The plant material was oven-dried (40 °C) up to a constant weight and coarsely powdered. The dried BP powder was accurately weighed, and sequential Soxhlet extraction was carried out with hexane, ethyl acetate, n-butanol, and distilled water. The extraction was continued with each solvent until the leachate become colorless and the subsequent solvents were added following the complete evaporation of the previous. The solvent extracts were collected separately. The organic extracts were concentrated by rotary evaporation (Buchi, B-480, UK) and dried in a vacuum (40 °C) to obtain the extracts of BP-hexane (BPH) (yield: 1.96% w/w), BP-ethyl acetate (BPEA) (yield: 6.51% w/w), and BP-n-butanol (BPNB) (yield: 5.47% w/w). The aqueous extracts were freeze-dried (−20 °C) to obtain the extract of BP-water (BPW) (yield: 9.32% w/w).

2.4. Preliminary phytochemical screening

The presence of bioactive phytoconstituents including phenolic compounds, tannins, flavonoids, steroid glycosides, alkaloids, terpenoids, coumarins, and saponins was tested in BPH, BPEA, BPNB, and BPW extracts according to previously published methods.[21]

2.5. Development of thin-layer chromatography (TLC) fingerprints

Thin-layer chromatography (TLC) profiles were developed for the selected extracts of BP using the solvent systems, which showed fine separations with a maximum number of components.[22] The solvent systems of dichloromethane:cyclohexane:methanol (1:1:0.1) and dichloromethane:cyclohexane:methanol:diethylamine (1:0.8:0.1:0.3) were used in the development of TLC fingerprints.

2.6. Gas chromatography-mass spectrometry (GC-MS) analysis

For further identification of the bioactive compounds, gas chromatography-mass spectrometry (GC-MS) analysis on hexane and ethyl acetate extracts of BP was carried out using an Agilent 6890 series gas chromatograph interfaced to the Agilent 5973 N series mass selective detector, which was equipped with a 5% phenyl methyl siloxane capillary column (HP-5 MS, 30 m × 0.25 mm ID, 0.25 μm film). Helium was used as the carrier gas and an injection volume of 1 μL was employed (split ratio of 10:1). The injector temperature was 270 °C. The oven temperature was programmed from 100 °C (5 min hold time) to 240 °C at a rate of 4 °C/min (40 min hold time). The samples were run for 40 min. The MS source and MS quadrupole temperatures were set at 230 °C and 150 °C, respectively. Parameters were scanned at 15–550 amu. The interpretation was performed using the Wiley W9N08.LIB database and NIST's.LIB.[23]

2.7. Determination of the total polyphenol content and the total flavonoid content

Total polyphenol and flavonoid contents of BPH, BPEA, BPNB, and BPW were determined following the standard protocols of the Folin-Ciocalteu method and aluminum chloride method respectively.[24,25] Quantification was carried out concerning the corresponding standard curves of gallic acid ($y = 3.1561x + 0.0269$) and quercetin ($y = 13.619x + 0.0024$). The results are expressed in terms of gallic acid equivalents of the dry weight of extract (mg GAE/g) and quercetin equivalents of the dry weight of extract (mg QE/g) for the total polyphenol and total flavonoid contents respectively.

2.8. Determination of 1,1′-diphenyl-2-picrylhydrazyl scavenging activity

The total antioxidant activity of the selected plant extracts was determined by 1,1′-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay.[26] Trolox was used as the reference compound and the results are expressed in terms of Trolox equivalents of the dry weight of the extract (mg trolox/g).

2.9. Experimental animals

The experimental procedures were carried out in strict accordance with the recommendations in the "Guide for the Care and Use of Laboratory Animals".[27] All procedures were reviewed and approved by the Ethical Review Committee of the Faculty of Medicine, University of Ruhuna (Permit Number: 14.12.2015:3.1). Blood collection and organ removal were performed under CO_2 euthanasia (EUTH 2A CO_2 euthanasia chamber, Orchid Scientific, India).

2.10. Experimental model of AKI

Freshly prepared DOX (Doxorubicin hydrochloride) at a single dose of 5 mg/kg in 0.9% saline (2500 μL/kg) was injected intraperitoneally into fasted (8 h) animals to establish the AKI model in Wistar rats. The dose of DOX was selected based on the literature and pilot experiments.[28,29]

2.11. Experimental protocol

Male Wistar rats weighing 150–175 g were randomly allocated into seven equally sized groups, with six animals in each group. Except for the control group (NC), DOX (5 mg/kg, ip) was injected into experimental rats in all other groups such as the model group, BPH treatment group, BPEA treatment group, BPNB treatment group, BPW treatment group, and FS treatment group to induce AKI. Twenty-four hours after DOX intoxication, treatment regimens were commenced. The human therapeutic dose (12 g/day for an adult human with 60 kg body weight) applied in Ayurveda medicine was extrapolated to experimental rats to calculate the relevant doses of plant extracts for administration based on the extraction yields of each extract and the dose conversion factor (6.17) from human to rat.[30] The BPH and BPEA extracts were suspended in corn oil for administration. BPNB was dissolved in 3% polyvinylpyrrolidone and BPW was dissolved in distilled water. All suspensions were stored at 4 °C. The experimental rats of treatment groups underwent oral gavage feeding of selected extracts of BP for four weeks at the equivalent human therapeutic dose of BPH (25 mg/kg/day), BPEA (80 mg/kg/day), BPNB (70 mg/kg/day), BPW (120 mg/kg/day) and fosinopril sodium (FS, 0.09 mg/kg/day). Normal control and model rats received an equivalent volume of distilled water. The plant extracts, fosinopril, and distilled water were administered in a volume of 0.2 mL/150 g using a stainless-steel oral gavage tube for 28 consecutive days. The duration of plant extract administration was defined considering the previously published reports on the nephroprotective potential of plant extracts against DOX-induced kidney injury.[31,32]

2.12. Blood sample collection and tissue dissection

Experimental rats were fasted (free access to water) before dissection. Twenty-four hours following the last dose of treatment, blood samples were collected by cardiac puncture, and serum was separated for the subsequent assessment of the kidney functions. The kidneys were removed from the sacrificed animals and bisected. One-half of each kidney was fixed in 10% formalin for the subsequent assessment of histopathology and immunohistochemistry. The remaining half was weighed and homogenized in ice-cold phosphate-buffered saline (0.01 M, pH = 7.4). Aliquots of kidney homogenates were stored at −80 °C for the determination of oxidative and inflammatory markers.

2.13. Biochemical analyses

Serum concentrations of creatinine, total protein, albumin, β_2-microglobulin, cystatin C, and blood urea nitrogen, were estimated according to the manufacturer's protocol, in the assessment of kidney function.

The test procedure supplied with each commercial kit was followed to estimate the total antioxidant status, glutathione reductase, and glutathione peroxidase for the assessment of oxidative stress in kidney homogenates. Lipid peroxidation was evaluated in terms of the concentration of malondialdehyde per gram of protein in kidney homogenates according to the thiobarbituric acid method. The inflammatory cytokines, tumor necrosis factor-α (TNF-α), and interleukin-1β (IL-1β) were quantified in kidney homogenates using ELISA kits for the assessment of anti-inflammatory markers.

2.14. Histopathological assessment

Hematoxylin and eosin-stained kidney sections were used in the morphological evaluation of features of AKI. The procedure in Bancroft and Gamble[33] was followed for the preparation of histology samples for hematoxylin and eosin staining. The histological changes were examined by two independent investigators including a consultant histopathologist who was blinded to the experimental profile. The morphological evaluation was carried out according to a semi-quantitative score system developed by the investigators, based on the features observed, as mentioned.

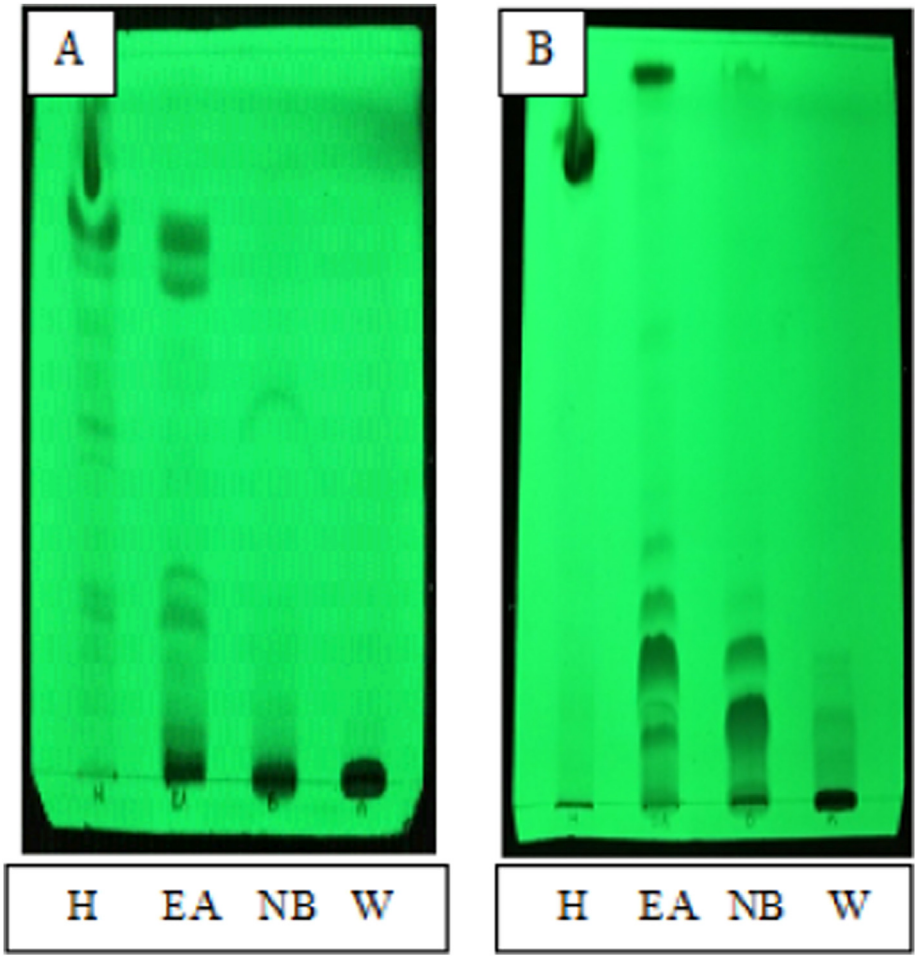

Fig. 1. Thin layer chromatography fingerprints of *Barleria prionitis* at UV 254 nm with the solvent systems; dichloromethane:cyclohexane:methanol (1:1:0.1) (A) and dichloromethane:cyclohexane:methanol:diethylamine (1:0.8:0.1:0.3) (B). H, EA, NB, and W, as mentioned in the Fig. were the hexane, ethyl acetate, n-butanol, and water extracts of *Barleria prionitis*, respectively.

Table 1
Thin layer chromatography of the selected extracts of *Barleria prionitis*.

Solvent system	Extract	No. of Spots	R_f values
Dichloromethane: Cyclohexane: Methanol (1:1:0.1)	BPH	17	0.04, 0.12, 0.15, 0.19, 0.22, 0.25, 0.38, 0.46, 0.47, 0.51, 0.59, 0.63, 0.69, 0.74, 0.84, 0.93, 0.97
	BPEA	11	0.06, 0.09, 0.15, 0.22, 0.26, 0.37, 0.44, 0.57, 0.68, 0.74, 0.96
	BPNB	04	0.51, 0.60, 0.75, 0.82
	BPW	–	
Dichloromethane: Cyclohexane: Methanol: Diethylamine (1:0.8:0.1:0.3)	BPH	13	0.05, 0.11, 0.18, 0.30, 0.43, 0.56, 0.63, 0.71, 0.80, 0.84, 0.88, 0.91, 0.95
	BPEA	12	0.09. 0.12, 0.17 0.26, 0.32, 0.39, 0.50, 0.54, 0.61, 0.86, 0.89, 0.96
	BPNB	07	0.06, 0.11, 0.19, 0.27, 0.62, 0.73, 0.96
	BPW	03	0.11, 0.16, 0.18

For each animal, 20 high-power fields (magnification: × 400) were examined in the kidney cortex as follows; tubular cell vacuolization, loss of tubular brush border, nuclear pyknosis, and glomerular congestion were quantified on a 4-point scale of 0 = none, 1 = < 25%, 2 = 25–50%, 3 = 50–75%, and 4 = >75% based on the area affected. The score for glomerular congestion was derived as the arithmetic mean of 100 glomeruli. Intertubular hemorrhage and inflammatory cell infiltrations were assessed by the absence (score = 0) or presence (score = 1) of the characteristic feature at high power (magnification: × 400). The presence of tubular casts was scored on a scale of 0–2 as 0 = none, 1 = 1 cast, and 2 = >2 casts. The overall histological score represents the sum of the mean values of observed changes for the comparison between the groups.

2.15. Immunohistochemical staining

Immunohistochemical staining was carried out on 4 μm thick sections of formalin-fixed, paraffin-embedded kidney tissue on poly-L-lysine-coated microscopic slides. Deparaffinised, rehydrated tissue sections were blocked for endogenous peroxidase activity and were incubated with proteinase K (Dako, S3020, Denmark) for antigen retrieval for anti-Bax (Abcam, ab216494, Cambridge, UK).

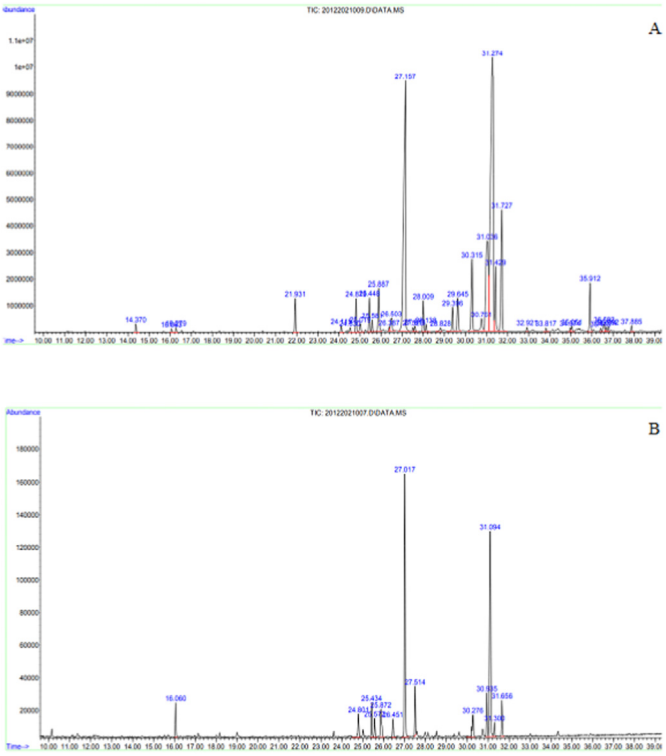

Fig. 2. The GC-MS chromatograms of the hexane (A) and ethyl acetate (B) extracts of *Barleria prionitis*.

Heat-induced antigen retrieval was carried out by microwave method (100 °C for 30 min) in citrate (pH 6) and Tris-EDTA (pH 9) buffers for anti-BCL-2 (Dako, M0887, Denmark) and anti-COX-2 (Dako, M3617, Denmark) respectively. After cooling and blocking, the tissue sections were incubated with the primary antibodies; Bax (1:100), BCL-2 (1: 25), and COX-2 (1:100) overnight (4 °C). Subsequently, the sections were incubated with horseradish peroxidase-conjugated (Dako REAL EnVision detection system, K4061, Denmark) secondary antibody (27 °C, 2 h) and counter-stained with hematoxylin. Brownish staining in the cellular cytoplasm under the light microscope was considered the positive staining in all three primary antibodies.

3. Statistical data analysis

Statistical analysis was performed using SPSS version 22. The samples were analyzed in triplicates. The biochemical parameters of the *in vivo* and *in vitro* studies were expressed as mean ± SD. Comparison of the same parameters among different groups was done using a one-way analysis of variance (ANOVA) followed by *post hoc* Fisher's least significant difference (LSD) multiple comparison tests. Pearson's correlation between the examined parameters was assessed. Statistical significance was assigned at $p < 0.05$.

4. Results

4.1. Screening of phytochemicals, TLC fingerprints, and GC-MS chromatogram of B. prionitis

TLC fingerprints were developed for the selected extracts of BP using two solvent systems based on the polarity of the extracts. The results of the analysis of TLC fingerprint profiles are presented in Fig. 1 and Table 1.

The preliminary phytochemical screening of BPH, BPEA, BPNB, and BPW revealed the presence of phenolic compounds. In contrast, alkaloids and coumarins were absent in all the tested extracts. Flavonoids and terpenoids were present in BPNB and BPW. In addition, tannins, steroid glycosides, and saponins were present in BPW.

The GC-MS chromatograms and the different bioactive compounds identified from BPH and BPEA are presented in Fig. 2A and B and Table 2, respectively.

4.2. Antioxidant activity of B. prionitis

Since reactive oxygen species and oxidative stress serve as the key factors in DOX-induced AKI, the total antioxidant potential of the four selected extracts of BP was assessed by DPPH radical scavenging assay *in vitro*. The total antioxidant activity through the DPPH assay was varied in order of BPNB > BPW > BPEA > BPH, as expressed by the equivalent antioxidant capacity of trolox, 53.24 ± 1.19, 29.23 ± 0.39, 11.19 ± 0.22, and 9.03 ± 0.23 mg trolox/g respectively. Extracts with organic solvents; BPH (18.71 ± 0.20 mg GAE/g), BPEA (22.83 ± 0.85 mg GAE/g), and BPNB (31.67 ± 0.18 mg GAE/g) showed a higher polyphenol content than that in the BPW extract (14.62 ± 0.21 mg GAE/g). Similarly, the respective organic extracts also showed a high flavonoid content (37.42 ± 1.22, 15.97 ± 0.17, and 9.06 ± 0.17 mg QE/g) compared to the BPW (5.64 ± 0.14 mg QE/g). Interestingly, as determined by the Folin-Ciocalteu method, the BPNB, which showed the highest antioxidant activity by DPPH assay, showed the highest polyphenol content.

4.3. Effect of B. prionitis on biochemical parameters of AKI

The effect of BP on biomarkers of AKI is shown in Fig. 3A–F. The BPW showed the highest nephroprotection among all the plant extracts considering both concentrations of creatinine (33%) and

Table 2
The chemical constituents identified from the hexane and ethyl acetate extracts of *Barleria prionitis*.

Name of the compound	Hexane extract		Ethyl acetate extract	
	Retention time (min)	Relative percentage (%)	Retention time (min)	Relative percentage (%)
Phenol, 2,4-bis(1,1-dimethylethyl)	16.066	0.14	16.054	3.50
Dodecanoic acid	16.279	0.19	–	–
Methyl tetradecanoate	21.928	1.36	–	–
3-Methylene-1-hexadecene	24.818	1.45	24.806	3.28
7-Octadecyne	–	–	25.434	3.84
2-Hexadecen-1-ol	25.884	2.11	25.872	4.47
Methyl palmitate	27.163	21.84	27.021	30.94
1-Dodecanol, 3,7,11-trimethyl-	30.764	1.09	–	–
9,12-Octadecadienoic acid (Z,Z)-	31.036	10.84	30.930	4.69
Cyclohexane	31.427	3.00	31.297	1.56
Palmitic acid	32.919	0.17	–	–

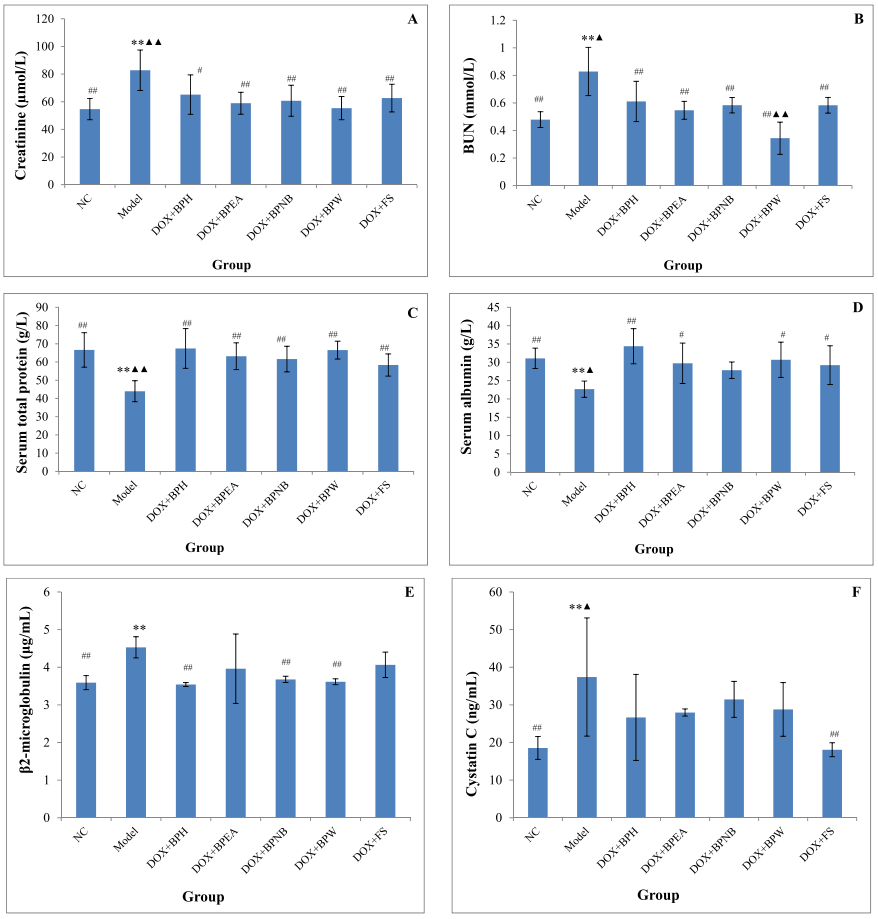

Fig. 3. Effect of *Barleria prionitis* extracts on the concentrations of serum creatinine (A); blood urea nitrogen (BUN) (B); serum total protein (C); serum albumin (D); β_2-microglobulin (E); serum cystatin C (F) in DOX-induced AKI. Data represent mean ± SD, n = 6. Compared with normal control group *p < 0.05, **p < 0.01; compared with model group #p < 0.05, ##p < 0.01 and compared with the FS treatment group ▲ p < 0.05, ▲▲ p < 0.01. NC: normal control, DOX: doxorubicin. The treatment groups (DOX + BPH, DOX + BPEA, DOX + BPNB, DOX + BPW, DOX + FS) consisted of experimental rats administered with hexane, ethyl acetate, n-butanol and water extracts of *B. prionitis* and fosinopril sodium, respectively.

blood urea nitrogen (58%). The blood urea nitrogen values of the experimental rats treated with BPW were significantly different from the BPH, BPEA, and BPNB as well as from fosinopril ($p < 0.05$). On the contrary, the experimental rats treated with FS (52%) showed a marked reduction in the concentration of cystatin C compared to the four extracts of BP (29%, 25%, 16%, and 23%).

4.4. Effect of B. prionitis on DOX-induced oxidative stress in kidney

Treatment with the selected extracts of BP for four consecutive weeks ameliorated DOX-induced oxidative stress significantly, by recovering the depleted total antioxidant status and the activity of glutathione reductase and glutathione peroxidase enzymes as shown in Fig. 4A–C ($p < 0.05$). The BPH (111%) and BPW (55%) demonstrated a significant improvement in glutathione peroxidase activity, compared to the NC rats ($p < 0.05$). Similarly, BPEA (40%) showed a significant increase in the glutathione reductase activity compared to the NC rats ($p < 0.05$). Notably, the experimental rats of the FS treatment group did not show significant improvement in the activity of glutathione reductase and glutathione peroxidase, compared to the DOX-induced model group in the present study ($p > 0.05$). Experimental rats in the BPEA (52%) and BPNB (29%) treatment groups showed a statistically significant increase in glutathione reductase activity, compared to the experimental rats in the FS treatment group ($p < 0.05$). Furthermore, there was a significant improvement in glutathione peroxidase activity in experimental rats from all four BP treatment groups, compared to the FS treatment group ($p < 0.05$).

However, all groups of experimental animals treated with BP showed a significant attenuation in malondialdehyde formation compared to the experimental animals of the model group (Fig. 4D) ($p < 0.05$). The experimental rats of the BPH and BPEA treatment groups showed a significant reduction in malondialdehyde formation, compared to the FS treatment group as well ($p < 0.05$). BPEA (30%) and BPNB (24%) showed the highest and lowest attenuation in lipid peroxidation, respectively. A significant correlation was observed in the activity of glutathione reductase and lipid peroxidation ($r = -663$, $p = 0.001$) in the present study.

4.5. Effect of B. prionitis on DOX-induced inflammation in kidney

Treatment with the plant extracts exhibited a significant suppression in the levels of TNF-α and IL-1β compared to the model group ($p < 0.05$). The results are shown in Fig. 4E–F. The BPW showed superior results over the other three extracts of BP concerning both TNF-α and IL-1β. A significant amelioration of TNF-α was observed with experimental rats in the BPW treatment group compared to the BPH, BPEA, and FS treatment groups ($p < 0.05$).

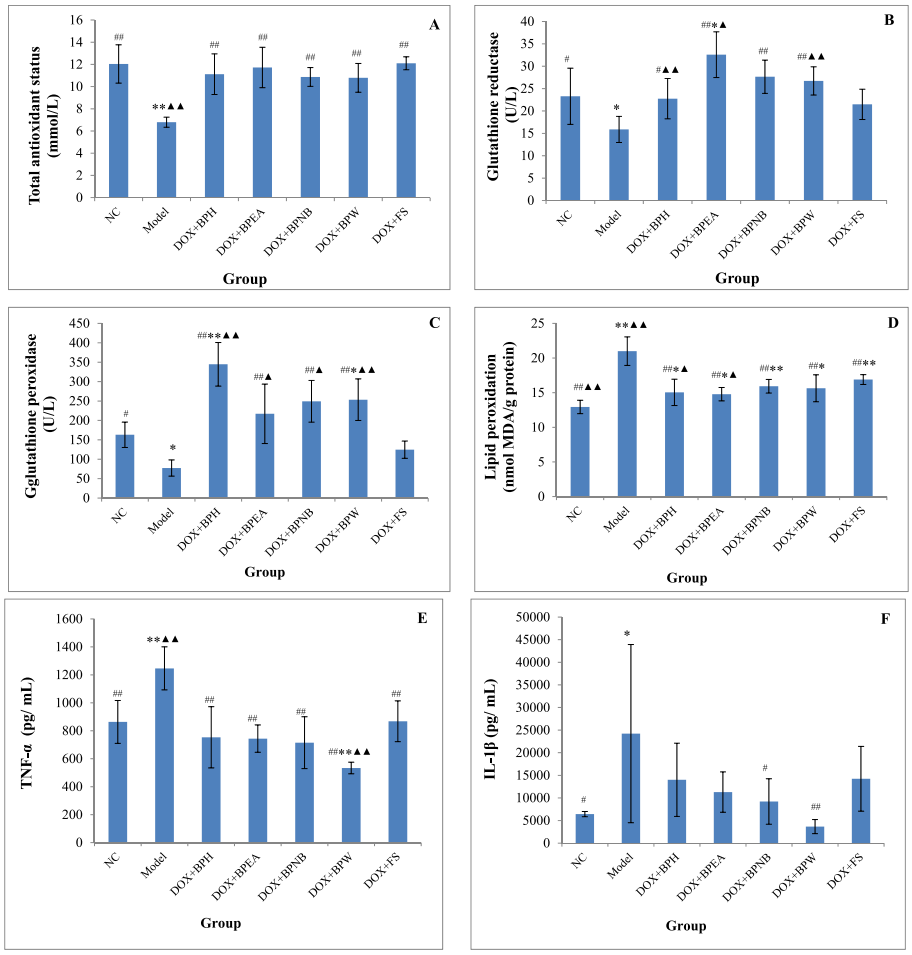

Fig. 4. Effect of *Barleria prionitis* extracts on total antioxidant status (A); the activity of glutathione reductase (B); glutathione peroxidase (C) and lipid peroxidation (D), concentration of tumor necrosis factor-α (TNF-α) (E) and interleukin-1β (IL-1β) (F) in kidney homogenates of DOX-induced AKI. Data represent mean ± SD, n = 6. Compared with normal control group * $p < 0.05$, **$p < 0.01$; compared with model group #$p < 0.05$, ##$p < 0.01$ and compared with the FS treatment group ▲ $p < 0.05$, ▲▲ $p < 0.01$. NC: normal control, DOX: doxorubicin. The treatment groups (DOX + BPH, DOX + BPEA, DOX + BPNB, DOX + BPW, DOX + FS) consisted of experimental rats administered with hexane, ethyl acetate, n-butanol and water extracts of *B. prionitis* and fosinopril sodium, respectively.

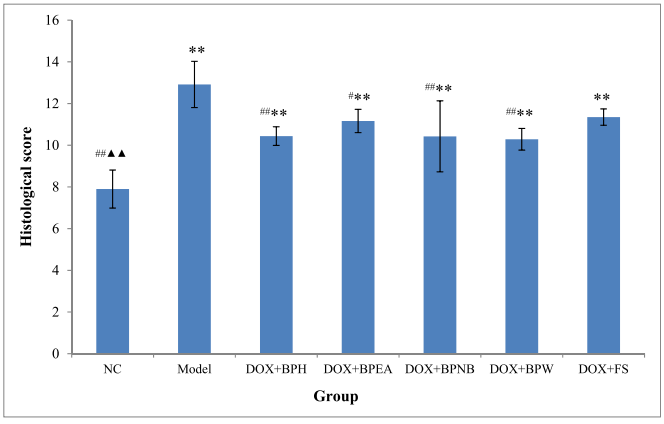

Fig. 5A. Effect of *Barleria prionitis* extracts on overall histological score of kidney sections stained with hematoxylin and eosin. Data represent mean ± SD, n = 6. Compared with normal control group * $p < 0.05$, **$p < 0.01$; compared with model group #$p < 0.05$, ##$p < 0.01$ and compared with the FS treatment group ▲ $p < 0.05$, ▲▲ $p < 0.01$. NC: normal control, DOX: doxorubicin. The treatment groups (DOX + BPH, DOX + BPEA, DOX + BPNB, DOX + BPW, DOX + FS) consisted of experimental rats administered with hexane, ethyl acetate, n-butanol and water extracts of *B. prionitis* and fosinopril sodium, respectively.

Interestingly, the anti-inflammatory activity of the four selected BP extracts was varied in the same order as BPW > BPB > BPEA > BPH considering both TNF-α, and IL-1β values.

4.6. Effect of *B. prionitis* on DOX-induced histopathological damage in the kidney

DOX administration resulted in marked kidney damage with an average score of 12.9, as mentioned in Fig. 5A, which was significantly attenuated by the treatment with BPH (19% decrease), BPEA (14% decrease), BPNB (19% decrease), and BPW (20% decrease) ($p < 0.05$). Histological examination showed cytoplasmic vacuolization, the disappearance of the brush borders, nuclear pyknosis, and hyaline casts in the renal tubules of the model group (Fig. 5B). However, no statistically significant improvement in the overall histological score was observed after treatment with fosinopril (12% decrease) ($p > 0.05$). The interstitial tissue showed very few lymphocytic aggregates at low power (× 100 magnification), however, it was difficult to quantify them at high power (× 400 magnification). Glomerular congestion and intertubular hemorrhage were the additional features observed. However, no evidence of full-blown acute tubular necrosis or glomerulosclerosis was observed in the kidney sections stained with hematoxylin and

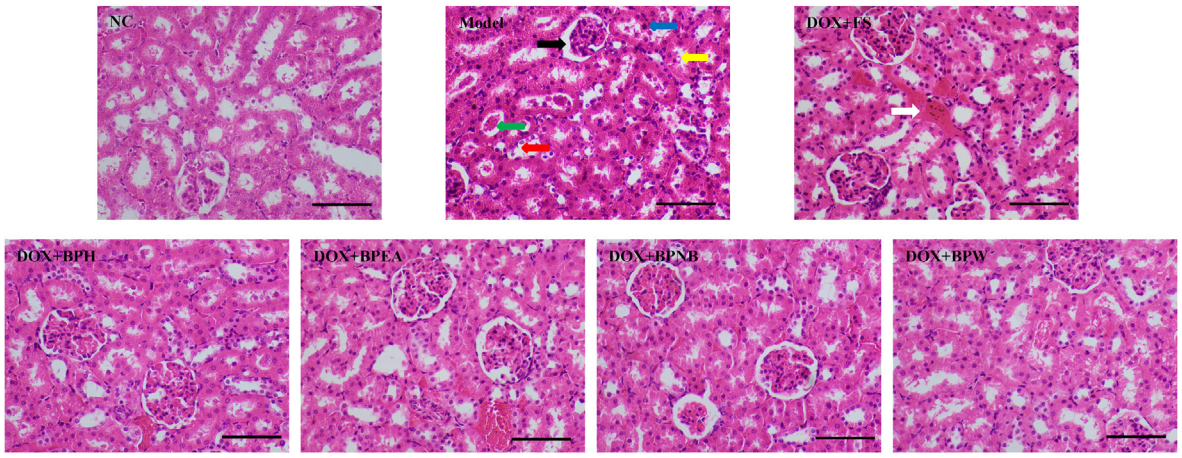

Fig. 5B. Effect of *Barleria prionitis* extracts on photomicrographs of kidney sections stained with hematoxylin and eosin (× 400 magnification, Scale bar: 20 μm) in DOX-induced AKI. Cytoplasmic vacuolization (yellow arrow), disappearance of the brush borders (red arrow), nuclear pyknosis (blue arrow), tubular casts (green arrow), glomerular congestion (black arrow) and intertubular hemorrhage (white arrow). NC: normal control, DOX: doxorubicin. The treatment groups (DOX + BPH, DOX + BPEA, DOX + BPNB, DOX + BPW, DOX + FS) consisted of experimental rats administered with hexane, ethyl acetate, n-butanol and water extracts of *B. prionitis* and fosinopril sodium, respectively.

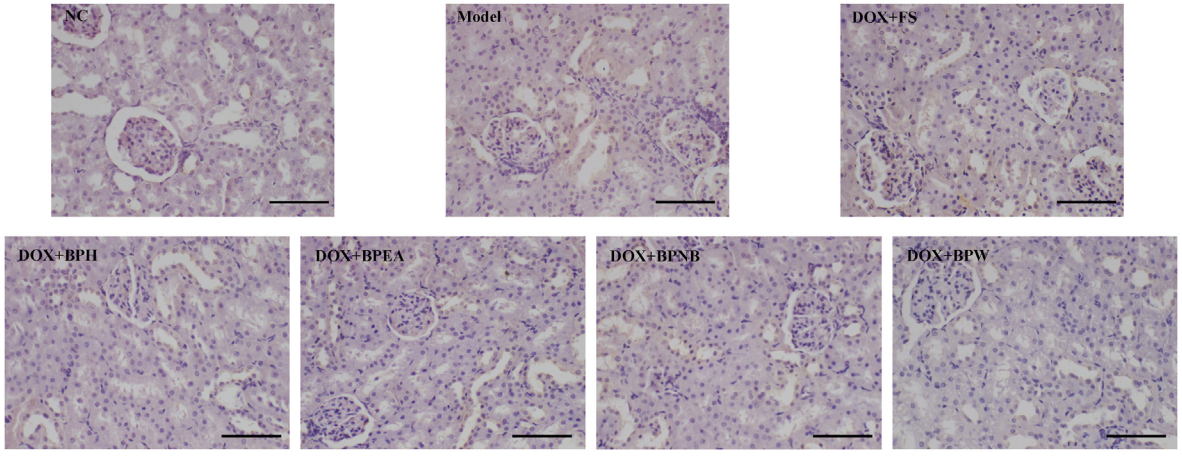

Fig. 6A. Effect of *Barleria prionitis* on the immunohistochemical expression of B-cell associated X protein (Bax) on DOX-induced AKI (× 400 magnification, Scale bar: 20 μm). The immunostaining of the pro-apoptotic protein, Bax was comparatively high and mainly cytoplasmic in the DOX-induced model group. Conversely, the staining was less and mainly visible in the luminal surface of the proximal and distal tubular epithelial cells, in the rats of NC group and most of the treatment groups. NC: normal control, DOX: doxorubicin. The treatment groups (DOX + BPH, DOX + BPEA, DOX + BPNB, DOX + BPW, DOX + FS) consisted of experimental rats administered with hexane, ethyl acetate, n-butanol and water extracts of *B. prionitis* and fosinopril sodium, respectively.

eosin in any of the experimental groups in the present study. Representative photomicrographs of kidney sections of the BP treatment groups are displayed in Fig. 5B.

4.7. Effect of B. prionitis on DOX-induced tubular cell apoptosis and inflammatory immuno-markers in kidney

The expression of the apoptosis-related proteins, BCL-2 and Bax were assessed by immunohistochemistry for the evaluation of the effect of selected plant extracts on DOX-induced apoptosis. The immunostaining of the pro-apoptotic protein, Bax, was comparatively high and mainly cytoplasmic in the DOX-induced model group (Fig. 6A). The staining was visible mainly on the luminal surface of the proximal and distal tubular epithelial cells in rats of the NC group and most of the treatment groups. Conversely, the expression of the anti-apoptotic protein; BCL-2 in tubular epithelial cells was comparatively high in rats of the NC group compared to the model group (Fig. 6B). An uniformly positive cytoplasmic expression of BCL-2 was observed in the NC group, whereas the focal positivity was observed in the model group rats. The experimental rats treated with plant extracts showed improved immunostaining for BCL-2 compared to the model group. Notably, the kidney sections of rats treated with BPH and BPW showed higher expression of BCL-2 and a lesser expression of Bax compared to the other two extracts. The anti-inflammatory potential of BP was assessed in terms of the immunohistochemical expression of COX-2 in the present study. Treatment with the selected BP extracts for four consecutive weeks caused a reduction in COX-2 expression. The BPW showed lesser expression of COX-2 compared to the other three extracts of BP in the present study. The immunohistochemical expression of COX-2 is shown in Fig. 6C.

5. Discussion

Numerous experimental studies conducted over the past few decades highlighted the value of phytomedicines as anticancer agents as well as adjuvants or alternative therapeutics in ameliorating the potential side effects of chemotherapeutics.[14] Protective

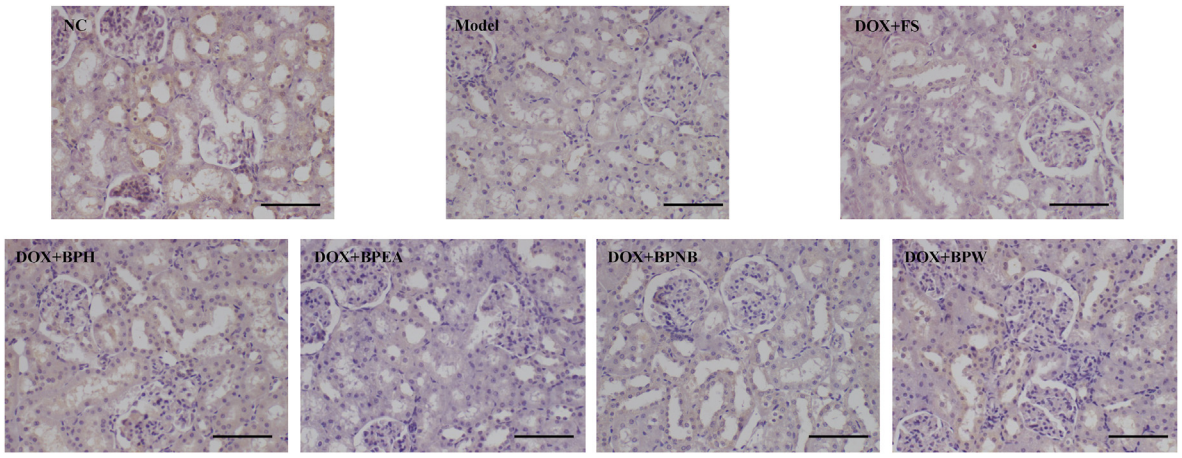

Fig. 6B. Effect of *Barleria prionitis* on the immunohistochemical expression of B-cell lymphoma gene product 2 (BCL-2) on DOX-induced AKI (× 400 magnification, Scale bar: 20 μm). The expression of the anti-apoptotic protein BCL-2 in tubular epithelial cells was comparatively high in rats of NC group compared to the model group. Uniformly positive cytoplasmic expression of BCL-2 was observed in the NC group whereas focal positivity was observed in the model group rats. The experimental rats treated with plant extracts showed enhanced immunostaining for BCL-2 compared to the model group. NC: normal control, DOX: doxorubicin. The treatment groups (DOX + BPH, DOX + BPEA, DOX + BPNB, DOX + BPW, DOX + FS) consisted of experimental rats administered with hexane, ethyl acetate, n-butanol and water extracts of *B. prionitis* and fosinopril sodium, respectively.

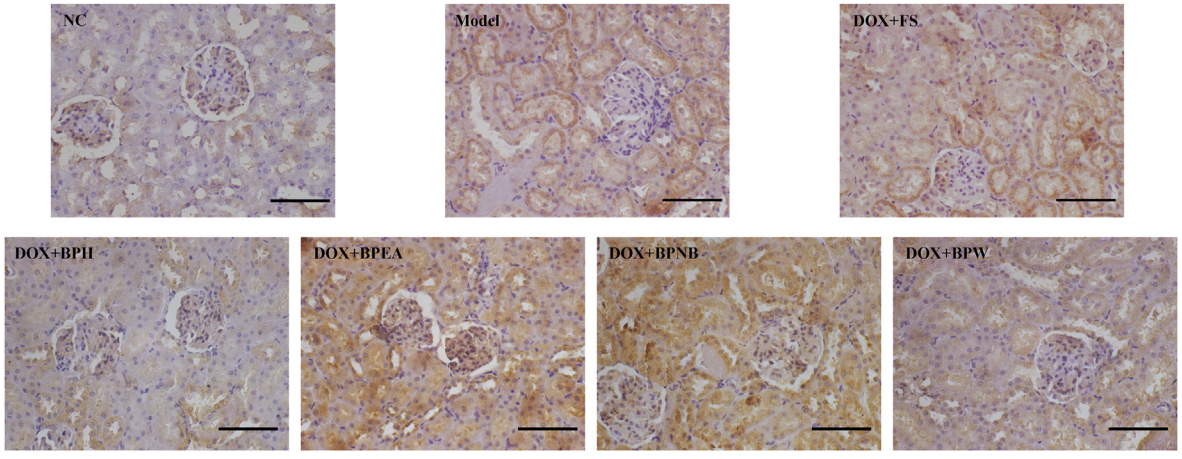

Fig. 6C. Effect of *Barleria prionitis* on the immunohistochemical expression of cyclooxygenase 2 (COX-2) on DOX-induced AKI (× 400 magnification, Scale bar: 20 μm). The immunohistochemical expression of COX-2 was comparatively higher in the experimental rats of the model group compared to the rats of the NC group. The treatment groups showed a reduction in the expression of COX-2 compared to the rats of the model group. NC: normal control, DOX: doxorubicin. The treatment groups (DOX + BPH, DOX + BPEA, DOX + BPNB, DOX + BPW, DOX + FS) consisted of experimental rats administered with hexane, ethyl acetate, n-butanol and water extracts of *B. prionitis* and fosinopril sodium, respectively.

mechanisms of phytomedicines in terms of antioxidative, anticarcinogenic, anti-inflammatory, immunoprotective, etc. were found to protect normal cells from chemotherapy-induced toxicities.[14] Treatment with the selected extracts of BP significantly ameliorated DOX-induced kidney injury, as shown by the improvements in kidney function biomarkers.

Histopathological findings corroborated the results of the biochemical parameters. None of the experimental groups showed a full-blown picture of acute tubular necrosis or glomerular sclerosis. Sub-lethal changes of AKI were observed in experimental rats of the model group which were ameliorated following the treatment regimens.

Oxidative stress is considered a major mechanism in the pathogenesis of DOX-induced AKI.[12] Excessive production of reactive oxygen species by exposure to DOX causes the corresponding generation of oxidative stress, including the depletion of reduced glutathione, the reduced level of antioxidant enzymes, and the increased levels of malondialdehyde.[11,12] The same phenomenon was observed in the present study; in fact, post-treatment with the selected extracts of BP effectively reversed DOX-induced oxidative stress to a normal degree. All four extracts of BP showed higher antioxidant potential than those of fosinopril, considering the activity of antioxidant enzymes; glutathione peroxidase, glutathione reductase, and the level of malondialdehyde. FS is an angiotensin-converting enzyme inhibitor and the use of the drug is a common therapeutic approach in chronic kidney disease due to its long-term nephroprotective effects.[34] In fact, angiotensin-converting enzyme inhibitors demonstrate hypoalbuminemia by inhibiting the renin-angiotensin system, thereby being the standard of care for patients with albuminuria.[35] Hence, fosinopril was used as the reference drug in the present study, considering its beneficial effects in ameliorating proteinuria associated with DOX-induced AKI. The potential nephroprotective effects of FS were further confirmed by the observed attenuation of proteinuria in the FS treatment group.

These findings on the enhancement of cellular antioxidant status by BP extracts are in line with previous reports which showed a potent antioxidant effect of BP.[16,17] According to published reports, numerous phytoconstituents isolated from BP, including balarenone, pipataline, lupeol, vanillic acid, melilotic acid, 6-

hydroxyflavone, β-sitosterol, verbascoside, etc. possessed antioxidant properties in the investigations of their potential bioactivities.[16,17] As inflammation is closely related to the generation of reactive oxygen species, anticancer drug-induced severe oxidative stress, generally induces inflammatory responses and *vice versa*. However, it is reported that the phytoconstituents with antioxidant potential could ameliorate inflammation-related side effects in chemotherapy.[14,36] The increased levels of TNF-α and IL-1β observed in the present study indicate an activation and increased production of pro-inflammatory cytokines in the kidney, leading to a state of inflammation in DOX-intoxicated rats. However, BP treatment significantly reversed DOX-induced inflammatory changes, suggesting that the nephroprotective effect of BP might occur partly due to its anti-inflammatory properties. The immunohistochemical findings on the expression of the inflammatory mediator, COX-2, were comparable to the findings on TNF-α and IL-1β. Accordingly, the present findings corroborate the anti-inflammatory effects of the aqueous extract of the plant.[37] Moreover, phytoconstituents isolated from BP including, lupeol, vanillic acid, 6-hydroxyflavone, β-sitosterol, p-hydroxybenzoic acid, verbascoside exerted anti-inflammatory potential.[16] The phytochemicals present in the BP extracts could inhibit the activity of enzymes that mediate inflammation, such as decreasing the damage caused by acute inflammation.[38] The presence of sub-lethal changes related to acute kidney injury and the absence of inflammatory infiltration in the present study are related to the potential propagation of apoptotic cell death rather than necrotic cell death. This was further corroborated by the immunohistochemical expression of the pro-apoptosis protein, Bax, and the anti-apoptosis protein, BCL-2. DOX administration caused up-regulation of Bax and down-regulation of BCL-2, promoting apoptosis, as demonstrated in the present study. The plant extracts showed better immunostaining for BCL-2 and relatively lower expression of Bax, suggesting the potential anti-apoptotic effects.

The present findings on the potential nephroprotective mechanisms of BPH, BPEA, BPNB, and BPW provide the basis for bioassay-guided isolation of nephroprotective phytoconstituents. Therefore, the present findings would open new vistas for the development of new nutraceuticals to minimize DOX-induced AKI in cancer patients.

6. Conclusions

The present study offered the first evidence that BPH, BPEA, BPNB, and BPW significantly ameliorate DOX-induced AKI via antioxidative, anti-inflammatory, and anti-apoptotic effects in experimental rats. The findings of *in vitro* antioxidant studies further substantiated the potential antioxidant effects of the plant extracts. The BP extracts might serve as a potential therapeutic/adjuvant for assisting DOX chemotherapy through ameliorating AKI in cancer patients who are on chemotherapy. However, further studies are required to confirm the clinical efficacy of the selected extracts in DOX chemotherapy.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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