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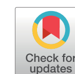
Protective effects of three selected standardized medicinal plant extracts used in Sri Lankan traditional medicine in adriamycin induced nephrotoxic Wistar rats



Sachinthi S. Amarasiri[a,**], Anoja P. Attanayake[b,*], Liyanagae D.A.M. Arawwawala[c], Kamani A.P.W. Jayatilaka[b], Lakmini K.B. Mudduwa[d]

[a] Department of Medical Laboratory Science, Faculty of Allied Health Sciences, University of Ruhuna, Galle, Sri Lanka
[b] Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka
[c] Industrial Technology Institute, Colombo, Sri Lanka
[d] Department of Pathology, Faculty of Medicine, University of Ruhuna, Galle, Sri Lanka

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ABSTRACT

Ethnopharmacological relevance: *Abelmoschus moschatus* Medik. (family: Malvaceae), *Asparagus falcatus* (family: Asparagaceae) and *Barleria prionitis* Linn. (family: Acanthaceae) have been used in the treatment of kidney diseases in Sri Lankan traditional medicine. Besides the traditional use, scientific scrutinization of safe therapeutic use of these medicinal plants in the management of kidney diseases has not been reported to date.

Aim of the study: The three selected doses of the aqueous extracts of the selected medicinal plants were studied for their protective effects against adriamycin (ADR) induced nephrotoxicity in Wistar rats.

Materials and methods: Chemically standardized plant materials were used in the study. The nephroprotective activity of the lyophilized powder of the aqueous refluxed (4hr) leaf extracts of *A. moschatus, A. falcatus* and the whole plant extract of *B. prionitis* was investigated in adriamycin (20 mg/kg, ip) induced nephrotoxicity in Wistar rats (n = 6/group). The treatment regimens were initiated 24 h after the induction of nephrotoxicity and continued daily as a single dose for three consecutive days at three selected doses (200, 400 and 600 mg/kg). Fosinopril sodium (0.09 mg/kg) was used as the standard drug. Nephroprotective activity was assessed by estimating the selected biochemical parameters and by the assessment of histopathology on H and E stained sections of the kidney.

Results: The plant extracts at the three selected doses significantly attenuated the elevations in serum creatinine, blood urea nitrogen and the loss of urine total protein in a dose related manner in ADR induced nephrotoxic rats ($p < 0.001$). The serum concentration of albumin and total protein increased significantly ($p < 0.001$). Histopathological findings corroborated the biochemical evidence of nephroprotective activity. The aqueous extracts of the three selected medicinal plants exerted a relatively high antioxidant activity *in vitro*.

Conclusions: Evaluation of the protective effects based on biochemical parameters and histopathology assessment revealed that the aqueous leaf extracts of *A. moschatus*, *A. falcatus* and the whole plant extract of *B. prionitis* possess significant nephroprotective activity against ADR induced acute nephrotoxicity. The secondary metabolites present in the plant extracts may attribute to the total antioxidant activities of the selected medicinal plant extracts thereby exerting protective effects against nephrotoxicity in Wistar rats.

1. Introduction

Sri Lankan traditional medicine systems as Ayurveda, Siddha, Unani and Deshiya Chikitsa were the mainstay of treatment in the country for variety of disease conditions during the ancient era where allopathic medicine systems were not grounded (Gunaratna et al., 2015; Weragoda, 1980). Even today, a considerable population of the country relies on these traditional systems of medicine in the management of

* Corresponding author. Department of Biochemistry, Faculty of Medicine, University of Ruhuna, Karapitiya, Galle, 80000, Sri Lanka.
** Corresponding author.
E-mail addresses: amssamarasiri@gmail.com (S.S. Amarasiri), anoja715@yahoo.com, anoja715@yahoo.com (A.P. Attanayake), menukaarawwawala@yahoo.com (L.D.A.M. Arawwawala), ayomawijewardena@yahoo.com (K.A.P.W. Jayatilaka), lakminimudduwa@yahoo.com (L.K.B. Mudduwa).

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List of abbreviations

ABTS	2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt
ADR	Adriamycin
Ang-II	Angiotensin II
BUN	Blood urea nitrogen
DPPH	2, 2′-diphenyl-2-picrylhydrazyl
H and E	Haematoxylin and eosin
IC_{50}	Concentration of the extract/reference compound at 50% inhibition
LC-MS	Liquid chromatography mass spectrometry
LD_{50}	Median lethal dose
LSD	Least significant difference
OECD	Organization for Economic Cooperation and Development
ORAC	Oxygen radical absorbance capacity
PPE	Personal protective equipment
SLS	Sri Lanka Standard
TEAC	Trolox equivalent antioxidant capacity
TLC	Thin layer chromatography
Trolox	6-hydroxy-2-5-7-8-tetramethylchroman-2-carboxylic acid
WHO	World Health Organization

chronic diseases such as diabetes mellitus, arthritis, liver, kidney diseases etc. (Abat et al., 2017; Muralitharan and Chandler, 2007; Perera, 2012).

Medicinal plants are the key elements in the traditional systems of medicine (Chaudhury and Rafei, 2001). They are used in various forms of preparations as powder, decoctions, oil etc., as a single herb or a mixture of herbs in therapeutic applications. Effectiveness, low cost, affordability and safety of the ethno medicinal preparations have made them popular among general public.

A large number of medicinal plants have been used in Sri Lanka, which claims therapeutic efficacy in kidney related diseases (Jayaweera, 1982). Among them, *Abelmoschus moschatus* Medik. (common name: Kapukinissa, English name: Musk mallow, family: Malvaceae), *Asparagus falcatus* L. (common name: Hathavariya, family: Asparagaceae) and *Barleria prionitis* L. (common name: Katukarandu, English name: Porcupine flower, family: Acanthaceae) are of particular interest in the treatment of kidney diseases mainly due to their documented diuretic effects (Jayaweera, 1982).

A. moschatus (Plate 1A) is a folk medicinal shrub with a long history of traditional uses for an array of diseases (Anil and Neeraj, 2017; Dwivedi and Argal, 2016, 2017). In Ayurveda, various parts of the plant as leaves, stem, seeds, and roots are used to pacify aggravated pitta, kapha, headaches, rheumatism, anorexia, varicose veins, fever, gonorrhea, dysuria and nervous system disorders (Jayaweera, 1982; Pawar and Vyawahare, 2016). The plant is given as "kashaya" or in the powder form as a coffee-like drink or with a vehicle similar to bee honey in traditional remedies (Ayurveda Pharmacopoeia, 1985). It is used in the preparation of the Ayurvedic herbal recipe called "dasamularistaya" as well. Especially, a decoction of pounded seeds are claimed to be useful in the management of renal calculi. Similarly, *A. falcatus* (Plate 1B) has been used in the treatment of variety of diseases in Sri Lankan traditional systems of medicine. It has a long history of being used as a folk medicine in the treatment of respiratory diseases, chronic congestion of liver, jaundice, gallstones, venereal diseases, diarrhoea, dysentery and kidney diseases (Hewawasam et al., 2008; Jayaweera, 1982). *A. falcatus* is a major constituent of the herbal porridge commonly known as "Kola Kanda" (rice based porridge) by Sri Lankans. It is used for the alleviation of vatha and pitta humours in Ayurveda. The value of *A. falcatus* in the treatment of dysuria is recognized in many Ayurveda text books (Ayurveda Pharmacopoeia, 1985; Jayaweera, 1982). It is recommended to consume half-a-teaspoon of the powdered tuber twice a day with cold water in the treatment of dysuria in Ayurveda (Ayurveda Pharmacopoeia, 1985). In addition, a decoction of the plant tubers prepared with milk called "kiri kashaya" is given for patients with haematuria.

B. prionitis (Plate 1C) has been used in the treatment of kidney related diseases in Sri Lankan traditional medicine. It is a major component in the Ayurvedic preparation including "Rasnadi kashaya", "Saptasaram kashaya" and the "Vathapy capsule" which are useful in the treatment of arthritis and other "vata" disorders, hip pain, menstrual pain and paralysis respectively. A decoction made of leaves or whole plant has been used in Ayurveda in the treatment of difficulty and burning sensation in urination (Ayurveda Pharmacopoeia, 1985).

ADR is an anthracycline antibiotic, widely used as a chemotherapeutic agent for the treatment of variety of malignancies including leukemia, lymphoma, several types of carcinomas and soft tissue sarcomas (Abdel-Daim et al., 2017; Abushouk et al., 2017, 2019). However, its clinical use has been limited due to its cumulative multi-organ toxicities including nephrotoxicity, cardiotoxicity, pulmonary, testicular and hematological toxicities (Ayla et al., 2011; Abdel-Daim et al., 2017; Abushouk et al., 2017, 2019). Even though, the exact mechanism of ADR induced nephrotoxicity is not clearly understood, it has been proposed that ADR metabolism may induce mitochondrial toxicities mediated through free radical formation, membrane lipid peroxidation, protein oxidation, impairment of calcium homeostasis and release of cytochrome C that may contribute to apoptosis or necrosis of

Plate 1. Photographs of *Abelmoschus moschatus* (A), *Asparagus falcatus* (B) and *Barleria prionitis* (C) grown in Sri Lanka in natural habitat.

glomerular and tubular cells (Ayla et al., 2011; Raza and Naureen, 2019). However, the administration of therapeutic agents derived from medicinal plants seems promising in attenuating ADR induced nephrotoxicity, preserving relatively normal kidney function as well as glomerular and tubular structure (Alam et al., 2013; Mohebbati et al., 2016; Taskin et al., 2014).

Despite the traditional use, scientific scrutinisation of therapeutic use of *A. moschatus*, *A. falcatus* and *B. prionitis* in the treatment of kidney diseases has not been reported to date. Herein, we report the nephroprotective effects of standardized aqueous leaf extracts of *A. moschatus*, *A. falcatus* and whole plant extract of *B. prionitis* Linn on ADR induced nephrotoxicity in Wistar rats through the estimation of selected biochemical parameters on renal function and semi quantitative assessment of histopathology on H and E stained sections of the kidney.

2. Materials and methods

2.1. Chemicals, reagents and instruments

Gallic acid (CID: 370), quercetin (CID: 5280343), 2,2′-diphenyl-2-picrylhydrazyl (CID: 74358), 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (CID: 24890599), 6-hydroxy-2-5-7-8-tetramethylchroman-2-carboxylic acid (CID: 40634) and dichloromethane (CID: 6344) were purchased from Sigma Chemical Co, USA and were used in *in vitro* experiments.

Adriamycin (doxorubicin hydrochloride) was purchased from United Biotech, India. The standard drug fosinopril sodium (CID: 23681451) and diethyl ether (CID: 3283) were purchased from Sigma Chemical Co, USA and were used in *in vivo* experiments. All other chemicals and solvents were of analytical grade and were used without any purification.

A Spectra max plus microplate spectrophotometer (USA) and a Spectra max Gemini EM microplate fluorescence reader (China) were used in the determination of total antioxidant activity of the selected medicinal plant extracts. A UV-1800, SHIMADZU recording double beam spectrophotometer (USA) was used in the spectrophotometric bioassays. Tissue processor (Shandon, UK), microtome (Thermo Fisher, Germany) and microscope (Olympus CX 21, Japan) were used in the preparation and observation of hematoxylin and eosin stained tissue sections of the kidney tissues in the histopathology assessment respectively.

2.2. Plant material

Leaves of *A. moschatus, A. falcatus* and whole plant of *B. prionitis* were collected from natural habitats of Southern and Western provinces of Sri Lanka. A composite sample of plant materials were used in the study. The botanical identity of the medicinal plants was confirmed by comparing with the authentic samples at National Herbarium, Royal Botanical Gardens, Peradeniya, Sri Lanka. Voucher specimens of the medicinal plants were deposited in the Mini Herbarium at Department of Biochemistry, Faculty of Medicine, University of Ruhuna (voucher specimen numbers; PG/2016/55/01, PG/2016/55/02 and PG/2016/55/03).

2.3. Preparation of plant extracts

The selected plant parts were washed under running tap water, cut into small pieces and dried in a hot air oven (40 °C) to a constant weight. The oven dried plant materials were ground to obtain the powdered form of the medicinal plants.

The powdered crude plant material was used in the determination of physicochemical parameters of the selected medicinal plants. Aqueous plant extracts with an initial concentration of 50 mg/mL (2.50 g of powdered plant material in 50 mL of distilled water) refluxed for 4 h were used in the preliminary screening of phytoconstituents and in the investigation of total antioxidant activity. The concentrated aqueous plant extracts were subjected to freeze drying at −20 °C to obtain the lyophilized powder to be used in the investigation of acute nephroprotective activity. The percentage yields (on dry weight basis) of *A. moschatus*, *A. falcatus* and *B. prionitis* were 25.75%, 27.04% and 27.18% respectively. The lyophilized powder of the plant material was then dissolved in distilled water for the preparation of the three selected doses.

2.4. Standardization of medicinal plants

Determination of physicochemical parameters including moisture content, total ash content, acid insoluble and water soluble ash contents, ethanol and water extractable matter was carried out to the oven-dried crude powder form of the selected medicinal plants according to the methods described in guidelines of WHO (WHO, 1998). In addition, determination of the heavy metal content and microbial contamination was conducted according to SLS standards (SLS, 1973; SLS, 1982; SLS, 1992).

Qualitative phytochemical screening was conducted to identify the presence of alkaloids (Wagner's test), tannins and phenolic compounds (Ferric chloride test), flavonoids (Shinoda test), steroids (Salkowski test), coumarins (Alcoholic KOH test), saponins (Foam test) and terpenoids (Salkowski test) (Dalhat et al., 2018; Hussain and Kumaresan, 2014; Mengane, 2016; Pawar and Vyawahare, 2016).

Thin layer chromatography (TLC) profiles were developed for the dichloromethane extracts of the selected medicinal plants. The solvent systems which showed fine separation with maximum number of components were selected for the development of TLC fingerprint.

Liquid chromatography mass spectrometry (LC-MS) patterns were developed for the crude extracts of the three selected medicinal plants. Each sample (20 μL) was injected into a SunFire TM, C-18 5 μm reverse phase column (3.0 mm × 150 mm) of a LC-MS system connected to a tandem mass spectrophotometer at 50–2000 Da mass range. The LC-MS analysis was performed using ammonium acetate (5 mM) and formic acid (0.1%) in water as the mobile phase with a gradient elution at a flow rate of 0.5 mL/min.

2.5. Quantitative determination of total polyphenol content and total flavonoid content

Total polyphenol content of the selected extracts was determined using Folin-Ciocalteu's method (Singleton et al., 1999). Quantification was done with respect to the standard curve of gallic acid in the range 0–1 mg/mL ($y = -0.0101x + 0.0203$). The results were expressed in gallic acid equivalents of the dry weight (mg GAE/g). Total flavanoid content was determined using aluminum chloride method described by Siddhuraju and Becker (2003). The flavonoid content was calculated using the calibration curve of quercetin in the range of 0–125 μg/mL ($y = 13.619x + 0.0024$). The results were expressed as mg quercetin equivalent/g of extract. The assays were performed in triplicates.

2.6. In vitro antioxidant studies

Total antioxidant activity of the lyophilized powder of the aqueous refluxed plant extracts of *A. moschatus*, *A. falcatus*, and *B. prionitis* were determined by 2,2′-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^+$) radical scavenging assay and oxygen radical absorbance capacity (ORAC) assay. 6-hydroxy-2-5-7-8-tetramethylchroman-2-carboxylic acid (Trolox) was used as the reference compound. The assays were performed in triplicates.

DPPH radical scavenging assay was performed by the method reported by Blois (1958). The decrease in absorbance values of DPPH on addition of plant extracts/reference compound in relation to the control (DPPH solution alone) was used to calculate the antioxidant activity, in

terms of IC_{50} (concentration of the extract/reference compound at 50% inhibition). Similarly, IC_{50} values were calculated for the plant extracts in $ABTS^+$ radical scavenging assay by the method described by Re et al. (1999). Trolox was used as the reference compound in DPPH and ABTS assays. The results were expressed in terms of trolox equivalent antioxidant capacity (TEAC). Oxygen radical absorbance capacity values of the plant extracts were calculated considering the results of area under curve with reference to the reference compound; trolox during the ORAC assay and the results were expressed in terms of trolox equivalent antioxidant capacity (TEAC) (Ou et al., 2001).

2.7. Screening of medicinal plant extracts for acute nephroprotective activity in rats with ADR induced nephrotoxicity

2.7.1. Selection of doses of plant extracts

The lyophilized powder of each of the three selected plant extracts was dissolved in distilled water in the preparation of the three selected doses of the plant extracts. The three doses were; 200, 400 (equivalent human therapeutic dose in rats) and 600 mg/kg. The equivalent human therapeutic dose in rats was calculated by extrapolating the therapeutic dose of herbal extracts used by the Ayurvedic practitioners in the management of kidney related diseases (Dhawan and Srimal, 1997).

2.7.2. Experimental animals

Healthy rats of Wistar strain (275 ± 25 g, 10–12 weeks of age), purchased from the Medical Research Institute, Colombo, Sri Lanka were used in the experiments. Both male and female rats were used in the acute oral toxicity study whereas male rats were used in the experiments on nephroprotective activity of selected medicinal plant extracts. The animals were housed in standard environmental conditions at the animal house of the Faculty of Medicine, University of Ruhuna, Sri Lanka. They were maintained on a standard laboratory diet of pellets and water *ad libitum*. The rats were allowed to acclimatize for a period of seven days under standard environmental conditions before the commencement of experiments. Ethical clearance was obtained from the Ethical Review Committee, Faculty of Medicine, University of Ruhuna, Sri Lanka (14.12.2015:3.1).

Experiments were carried out with minimum pain, suffering or distress to the experimental animals. This was achieved by improving animal husbandry and housing, careful handling of the animals and exposure to appropriate anesthetic agents during experiments etc. Precautions were taken during handling of the chemicals/drugs. Personal protective equipment (PPE) including double nitrile gloves, cytotoxic safety goggles, lab coat and masks were used especially during the handling of the cytotoxic drug, ADR. Further, safe waste disposal procedures were applied during the study.

2.7.3. Acute oral toxicity study

Acute oral toxicity assessment was carried out according to the Organization for Economic Cooperation and Development (OECD) guideline 423 and fixed dose procedure (OECD, 2001). Healthy Wistar rats were randomly divided into four groups (three animals of each sex was used in each group/sub group), considering the average weight of animals. Group 1 (n = 6/group), administered with equivalent volume of distilled water once a day orally, served as the normal control. Animals of Group 2 to 4 were further divided into three sub groups (a-c; n = 6/sub group) and the lyophilized powder of the aqueous extracts of *A. moschatus, A. falcatus* and *B. prionitis* were orally administered at three selected doses (200, 400 and 600 mg/kg body wt.) respectively. The animals were observed for 3 h and then daily for 14 days for signs of toxicity. The animals observed for changes in skin, fur, eyes and in mucous membranes, respiration, tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.

2.7.4. Experimental induction of nephrotoxicity

A single intraperitoneal dose of ADR at 20 mg/kg was used in the induction of nephrotoxicity to Wistar rats.

2.7.5. Investigation of nephroprotective activity in ADR induced nephrotoxic rats

Overnight fasted (8h) healthy male Wistar rats were randomly divided into six groups. The first (n = 6/group) and the second groups (n = 6/group) served as the healthy and ADR induced nephrotoxic control groups respectively and received distilled water. Group three to five (for the three selected plant extracts separately) consisted of three sub groups (a-c; n = 6/sub group) and the ADR induced nephrotoxic rats were administered with the three selected doses of the lyophilized powder of the aqueous plant extracts orally. The sixth group (n = 6/group) which served as the positive control was administered with the standard drug fosinopril (0.09 mg/mL/kg b. wt.). The treatment regimens were initiated 24 h following the induction of nephrotoxicity and continued daily as a single dose for three consecutive days.

The body weight of experimental animals was measured on day one and five of the experiments. The percentage change in body weights per group was calculated. Consumption of food and intake of water were measured daily during the intervention.

Rats of each group were individually housed in metabolic cages at the end of the period of intervention and 24 h urine samples were collected. Rats were sacrificed under an exposure of an overdose of diethyl ether on the 5th day of the study and blood samples were collected into serum collecting gel tubes by cardiac puncture using 21G needle mounted on 3.0 mL syringe. Samples were left at room temperature (27 °C) for 1 h for coagulation prior to centrifugation. Then samples were centrifuged at 3500 rpm for 15 min for the separation of serum. Both serum and urine samples were stored at −70 °C until the samples were used in biochemical assays. Kidney tissues from the sacrificed rats were excised and fixed in 10% formalin for the preparation of haematoxylin and eosin (H and E) stained sections for the assessment of histopathological changes upon the treatment.

2.7.6. Biochemical analysis

Serum concentrations of creatinine (Biorex, UK), blood urea nitrogen (Biorex, UK), total protein (Stanbio, USA), albumin (Stanbio, USA) and urine total protein (Stanbio, USA) were estimated with commercially available test kits by spectrophotometric methods.

2.7.7. Assessment of histopathology

The kidney tissues were processed and embedded in paraffin. The sections were cut at a thickness of 4 μm. The sections were deparafinized, hydrated, and stained with haematoxylin and eosin (H and E) for the assessment of treatment related histological changes. H and E stained sections of kidney were examined under light microscope for the presence of indicators of cellular damage by two independent investigators of the study including a consultant histopathologist. The investigators were blinded to the experimental groups to which the histological sections belonged during the assessment of histopathology. Ten high power fields in the middle part of the kidney cortex of the H and E stained kidney sections were examined for the early features of acute tubular necrosis; tubular vacuolization, loss of brush border and nuclear pyknosis in renal tubular epithelium. These are the standard histopathological features which is dictate cell injury. Intertubular haemorrhages, glomerular congestion and formation of hyaline casts which are considered as the associated features were also observed. A semi quantitative scoring system developed by the investigators was used to assess the histopathological changes in the kidney. Scoring was done as follows: no damage = 0, presence of above mentioned features (each feature separately) = 1. The mean score value was calculated separately for each group of animals.

2.8. Statistical analysis

Data were statistically analysed using SPSS software 22.0 for

Windows. Quantitative data were expressed as mean ± SD. One-way ANOVA followed by the least significant difference (LSD) test was used for multiple comparisons of biochemical data in the *in vivo* study. The Kruskal- Wallis test was used for the analysis of semi quantitative data obtained in the assessment of histopathological changes. The values of $p < 0.05$ were considered statistically significant.

3. Results and discussion

In the present study, the nephroprotective activity of the aqueous extracts of *A. moschatus, A. falcatus* and *B. prionitis* at three selected doses was evaluated in ADR induced nephrotoxic Wistar rats.

3.1. Standardization of the selected medicinal plants

It is important to assure the quality of medicinal plant material, prior to be used them in bioactivity studies and in clinical applications. Hence, standardization of the selected medicinal plants was carried out in terms of determination of physiochemical constants, determination of heavy metal content and microbial contamination, identification of phytochemicals and development of TLC and LC-MS profiles. Table 1 shows the findings in physicochemical properties and heavy metal content of *A. moschatus*, *A. falcatus* and *B. prionitis* used in the study.

Ash values are the main indicators of contamination of plant material and are important in the assessment of purity and quality of herbal products (Kumari and Kotecha, 2016). The acid insoluble ash specifies the contamination of plant material from siliceous materials like earth and sand and low values in the present study denote the satisfactory purity of the plant material used. The water soluble extractives were found to be higher than the alcohol soluble extractives in all three selected medicinal plants in both cold and hot extracts. The hot water extractive values were the highest among the all extractives. These findings praise the use of hot water extracts in the *in vivo* experiments in the present study. The quantitative analysis of heavy metals revealed that the heavy metals including arsenic, lead, cadmium and mercury are present within the WHO acceptable limits (WHO, 2007). Moreover, no microbial contamination was detected in the plant materials selected. These findings indicate the purity of the plant material used in the study.

Phytoconstituents present in medicinal plants are the chemical compounds responsible for their therapeutic potential. Most of them exert antioxidant properties which may protect cells against oxidative stress and reduce the risk of occurrence of various oxidative diseases (Abat et al., 2017; Khan et al., 2010). Qualitative analysis of phytochemicals revealed that all three medicinal plants have common phytoconstituents as tannins, phenolics, flavonoids, steroid glycosides, terpenoids and saponins. Alkaloids were absent in all three extracts and coumarins were present only in the leaf extract of *A. falcatus.* The bioactive compounds such as flavonoids, tannins, saponins are reported with antioxidant and anti-inflammatory properties (Khan et al., 2010). Especially tannins present in herbal products are well known to possess antioxidant as well as antiuremic properties and hence they are important as nephroprotective agents (Alam et al., 2005). However, the findings of the phytochemical studies are not in complete agreement with previous data and this might be due to the geographical variations of the plant origin, seasonal variations, climatic conditions etc. (Talukdar et al., 2015; Christina and Muthumani, 2012).

Quantitative estimation of flavonoids and polyphenols of the selected medicinal plant extracts were carried out in accordance to the previously published methods. As given in Table 2, the results varied according to the order of *B. prionitis* > *A. falcatus* > *A. moschatus* for both total polyphenol content and total flavonoid content.

Qualitative chromatographic analysis of dichloromethane extracts of medicinal plants was done using thin layer chromatography to separate and identify the constituents present in the extracts. The details of the thin layer chromatography of dichloromethane extracts of the selected medicinal plants are shown in supplementary file 1, Table 1.The TLC fingerprint profiles were further supported with the LC-MS patterns of the three selected medicinal plants in the present study. The results are given in Plate 2. These fingerprint profiles would be beneficial in excluding the adulterants and in maintaining the quality and consistency of the selected plant material in future pharmaceutical interventions (Bijauliya et al., 2017).

3.2. In vitro antioxidant studies

Oxidative stress contributes to the development and progression of kidney diseases and its complications (Ruiz et al., 2013; Small et al., 2012). However, antioxidants in medicinal plants play a major role in the defense against oxidative damage via protecting cellular biological functions (Khan et al., 2009). Therefore, total antioxidant activity of the aqueous extracts of the three selected medicinal plants was systematically assessed in the present study.

Generally, antioxidant potential of natural products as crude extracts is not determined based on a single assay *in vitro* (Kaul et al., 2010). It is recommended to use at least three methods to determine the total antioxidant activity of plant materials considering the variations that could occur due to differences in reaction mechanisms and reaction conditions of different assay protocols (Boligon et al., 2014). However, assay procedures applied on free radical scavenging activity are

Table 1
Physicochemical analysis and quantitative estimation of heavy metals in *Abelmoschus moschatus* Medic. (leaves), *Asparagus falcatus* (leaves) and *Barleria prionitis* Linn. (whole plant).

Specification	*A. moschatus*	*A. falcatus*	*B. prionitis*
Ash values			
1. Total ash (% w/w)	17.19 ± 0.12	8.88 ± 0.14	15.4 ± 1.00
2. Acid-insoluble ash (% w/w)	1.79 ± 0.15	< 0.01	< 0.01
3. Water soluble ash (%w/w)	5.06 ± 0.52	6.38 ± 0.41	5.54 ± 0.49
Extractive values			
1. Cold water extractive (% w/w)	4.03 ± 0.54	3.77 ± 0.22	4.88 ± 0.96
2. Cold ethanol extractive (% w/w)	1.17 ± 0.31	1.11 ± 0.11	1.50 ± 0.31
3. Hot water extractive (% w/w)	7.24 ± 0.27	7.10 ± 0.13	6.81 ± 0.14
4. Hot ethanol extractive(% w/w)	1.98 ± 0.02	2.60 ± 0.10	3.64 ± 0.14
Moisture content (% w/w)	8.85 ± 0.27	7.96 ± 0.24	10.76 ± 0.07
Determination of heavy metals			
1. Mercury (Not more than 0.2 ppm)	Not detected	Not detected	Not detected
2. Arsenic (Not more than 5.0 ppm)	0.15 ppm	Not detected	Not detected
3. Lead (Not more than 10 ppm)	0.3 ppm	0.5 ppm	0.6 ppm
4. Cadmium (Not more than 0.3 ppm)	0.08 ppm	Not detected	Not detected

Values are expressed as mean ± SD, n = 3.

Table 2
Total antioxidant capacity of selected medicinal plants.

Plant	Total antioxidant capacity			Total polyphenol content (mg gallic acid/g of extract)	Total flavonoid content (mg quercetin/g of extract)
	DPPH assay (mg trolox/g of extract)	ABTS assay (mg trolox/g of extract)	ORAC assay (mg trolox/g of extract)		
A. moschatus	15.99 ± 0.64	73.02 ± 0.65	94.25 ± 13.03	20.88 ± 2.37	2.69 ± 1.87
A. falcatus	27.92 ± 0.41	134.14 ± 2.91	108.46 ± 14.72	33.49 ± 0.51	3.53 ± 0.25
B. prionitis	148.83 ± 2.11	182.68 ± 5.37	111.83 ± 7.76	48.32 ± 0.64	10.97 ± 2.06

comparatively straight forward to perform (Alam et al., 2013). Hence, DPPH, ABTS and ORAC assays were selected in the present study to determine the total antioxidant activity of the selected medicinal plants. According to Alam et al. (2013), these methods have used to ascertain the antioxidant potential of medicinal plant extracts in a number of scientific reports. The results of the three *in vitro* assays in terms of trolox equivalent antioxidant capacity (mg trolox/g of extract) are given in Table 2.

The DPPH radical scavenging assay is widely used in the determination of antioxidant potential of medicinal plants (Shi et al., 2010). It is a simple, rapid and relatively inexpensive method. This assay is based on the reduction of purple chromogenic radicals to a yellow coloured non radical form of DPPH in the presence of hydrogen donating antioxidants. A decrease in the absorbance of DPPH radical at 517 nm is proportional to the free radical scavenging potential of the plant extracts. The antioxidant activity is expressed in terms of IC_{50}; the concentration of the antioxidant required for a decrease in the percentage of inhibition by 50% of the initial DPPH concentration. A low IC_{50} value represents high level of antioxidant activity and *vice versa* (Boligon et al., 2014; Chetan et al., 2011). The total antioxidant activity of the three selected medicinal plants, determined by DPPH radical scavenging assay, was in the descending order of *B. prionitis, A. falcatus* and *A. moschatus* respectively. The values for *B. prionitis* in the present study is comparable with the results reported by Chetan et al. (2011), even though the aqueous leaf extract of *A. moschatus* presented relatively low values compared to the findings of Gul et al. (2011).

The ABTS method measures the loss of colour when an antioxidant is added to the blue- green chromophore $ABTS^{+}$ radical (2, 2- azino-bis (3-ethyibenzothiazolin-6-sulfonic acid)) reducing $ABTS^{+}$ to ABTS (Alam et al., 2013). The antioxidant activity is expressed in terms of IC_{50}; the concentration of the sample/reference compound at 50% inhibition. The results obtained for ABTS assay of *B. prionitis* in the present study is also comparable with the results reported by Chetan et al. (2011). Studies have not been reported for the antioxidant activity by ABTS assay for the other two plants.

ORAC is especially used for the determination of antioxidant power of edible medicinal plant extracts (Alam et al., 2013). The assay is based on generation of free radicals using AAPH (2,2-azobis (2-amidinopropane) dihydrochloride) and measurement of decrease in fluorescence in the presence of free radical scavengers. The antioxidant activity in

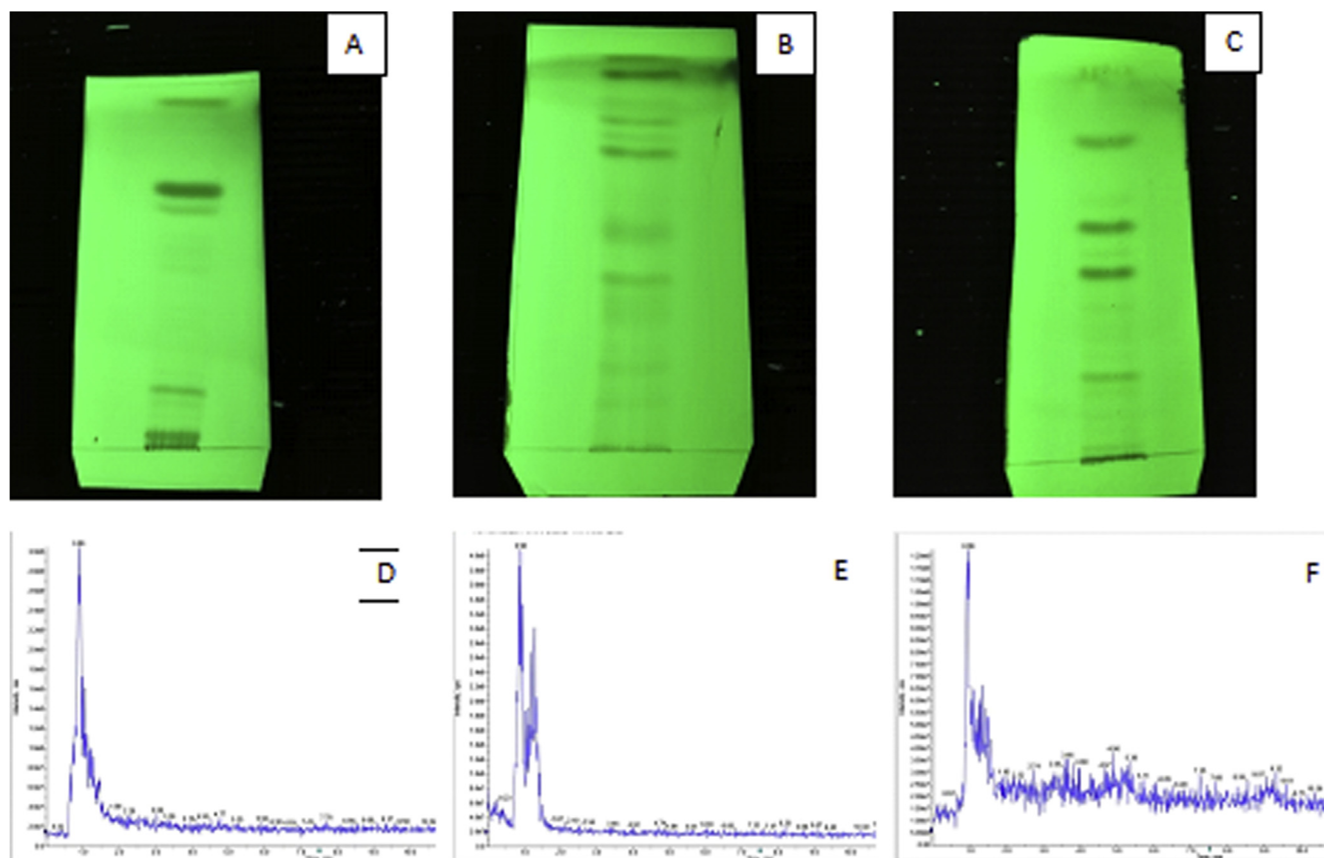

Plate 2. TLC Profiles for dichloromethane extracts (A, B, C) and LC-MS patterns for the crude plant extracts (D, E, F) of *Abelmoschus moschatus* (leaves), *Asparagus falcatus* (leaves) and *Barleria prionitis* (whole plant).

terms of ORAC value was calculated from Trolox (reference compound) equivalents (Alam et al., 2013). A high trolox equivalent antioxidant capacity (TEAC) value represents high antioxidant potential. Similar to other two assays, the aqueous extract of *B. prionitis* exhibited the most significant antioxidant activity by ORAC assay. Hence, the antioxidant activity is varied as *B. prionitis* > *A. falcatus* > *A. moschatus* in all three assays.

Even though it is difficult to compare different methods of antioxidant assays, by calculating the TEAC values, a comparison was made in the present study. Trolox is a water soluble analog of vitamin E and is being widely used as the reference compound in antioxidant assays performed in the aqueous phase (Alam et al., 2013). The TEAC values in DPPH and ABTS assays were calculated by comparing the IC_{50} values of medicinal plants with the IC_{50} value of the reference compound.

Values are expressed as mean ± SD, n = 3. Data are expressed as milligrams of trolox equivalents per gram of extract for antioxidant assays, milligrams of gallic acid equivalents per gram of extract for total polyphenol content and milligrams of quercetin equivalents per gram of extract for total flavonoid content.

These findings corroborated well with high polyphenol and flavonoid contents of the selected medicinal plant extracts which might be attributed to an increase in the antioxidant activity *in vitro*. The results obtained for total polyphenol and total flavonoid contents of the three selected medicinal plants varied according to the same order as total antioxidant activity i.e. *B. prionitis* > *A. falcatus* > *A. moschatus.* However, studies have not been reported for the antioxidant activity of *A. falcatus* and we report the total antioxidant activity for the first time.

3.3. Nephroprotective activity of the selected plant extracts on ADR induced nephrotoxicity in Wistar rat model

In search of novel therapeutic agents, animal models remain the gold standard for the investigation of beneficial bioactivities and the potential toxicity of the therapeutic agents prior to clinical trials (Ouedraogo et al., 2012). Among different *in vivo* models used in the screening of ethno medicinal plants with suggested nephroprotective activity, rodent models play a leading role (Ahmad et al., 2014; Council of the Royal Society, 2004). Accordingly, the Wistar rat model has been used in number of *in vivo* studies to assess nephroprotective activity of medicinal plants (Kannapan et al., 2010; Konda et al., 2016; Shelke et al., 2009; Hussain et al., 2012).

ADR induced nephrotoxicity in rats is one of the widely used chemically induced nephrotoxicity models in the evaluation of nephroprotective activity of medicinal plants. Several mechanisms are suggested for nephrotoxicity induced by ADR. The ADR toxicity is initiated with an accumulation of ADR in renal proximal tubular cells, especially in nuclei and in mitochondria causing mitochondrial degeneration and dysfunction (Malarkodi et al., 2003; Taskin et al., 2014). The impaired mitochondrial function may be contributed to an inhibition of oxidative phosphorylation leading to an impairment of energy metabolism, inhibition of electron transport complexes and disruption of calcium homeostasis (Taskin et al., 2014). The renal side effects of ADR are mainly attributed to the generation of reactive oxygen radicals. Redox cycling of the quinone functional group of ADR which triggers a status of oxidative stress is proposed as the key mediator in ADR induced nephrotoxicity. Production of reactive oxygen species may cause oxidative damage to various biological macromolecules and lipid membrane structures (Szalay et al., 2015). Further, adriamycin induces apoptotic cascades through two major signaling pathways, the mitochondrial pathway and the death receptor pathway. As mentioned earlier, mitochondrial pathway is the major signaling pathway which occurs in ADR induced toxicities (Ibrahim et al., 2019). Mitochondrial dysfunction releases mitochondrial cytochrome C into the cytoplasm, resulting in the formation of a complex of cytochrome C, Apaf-1, and caspase-9 enzyme, which is called an apoptosome. Apoptosome activates caspase-3, the leading protease in cell death. However, it is revealed that, activation of apoptotic pathways mediated by ADR induced oxidative stress has a critical role in cellular toxicity (Ibrahim et al., 2019). Moreover, it is confirmed that ADR generates various pro-inflammatory mediators such as cyclooxygenase-2, which can stimulate inflammatory pathways and play an important role in ADR induced nephrotoxicity (Ibrahim et al., 2019). In addition, ADR treatment results in an activation of renin angiotensin system in which Angiotensin II (Ang-II) plays a key role in the process of nephrotoxicity. Ang-II is able to increase oxidative stress by stimulating the generation of reactive nitrogen and oxygen species (El-shitany et al., 2008; Malarkodi et al., 2003; Taskin et al., 2014). Hence, ADR induced nephrotoxicity results in significant reduction in the antioxidant capacity of the kidney with a subsequent susceptibility to oxidative stress causing nephrotoxicity (Yilmaz et al., 2006).

Fosinopril is used in the present study as the standard drug. It is an angiotensin-converting enzyme inhibitor which is commonly used in the treatment of chronic kidney disease due to its long term nephroprotective effects (Navis et al., 1996; Qi et al., 2012). Traditional Ayurvedic practitioners primarily use the aqueous form of herbal preparations in their treatment. Accordingly, the aqueous extracts of the selected medicinal plants were administered to experimental animals in the present study. The high solubility of phytochemicals and the least toxicity of the aqueous extracts among variety of solvents used in the extraction of plant materials were the additional advantages of using aqueous extracts (Zahin et al., 2009).

3.3.1. Acute oral toxicity study in healthy Wistar rats

No mortality or toxicity was observed for the selected doses (200, 400 and 600 mg/kg) of the medicinal plant extracts in the 14 days of the study period. The results suggest that, the median lethal dose (LD_{50})

Table 3

Effect of selected plant extracts on renal biochemical parameters.

Treatment	BUN (mmol/L)	Serum Creatinine (μmol/L)	Serum Total protein (g/dL)	Serum Albumin (g/dL)	Urine Total protein (g/dL)
Healthy control rats	5.06 ± 0.65[c]	39.78 ± 18.59[c]	64.07 ± 4.50[c]	34.85 ± 1.15 [c]	83.86 ± 12.65[c]
ADR induced nephrotoxic control rats	8.87 ± 1.96	100.48 ± 27.05	47.23 ± 2.39	23.18 ± 0.86	304.99 ± 37.17
A. moschatus 200 mg/kg	5.82 ± 1.02[b]	77.35 ± 17.22[a]	54.68 ± 4.09[a]	23.28 ± 0.81	150.35 ± 43.61[c]
A. moschatus 400 mg/kg	4.70 ± 2.65[c]	58.34 ± 1.77[c]	54.78 ± 2.68[a]	25.75 ± 0.21[b]	136.00 ± 23.74[c]
A. moschatus 600 mg/kg	4.39 ± 1.37[c]	60.33 ± 5.51[c]	56.65 ± 2.21[b]	25.57 ± 1.36[a]	53.22 ± 23.68[c]
A. falcatus 200 mg/kg	6.98 ± 0.70[a]	68.07 ± 11.08[b]	52.70 ± 3.26	23.90 ± 1.27	281.55 ± 31.80
A. falcatus 400 mg/kg	5.45 ± 0.83[c]	49.28 ± 5.47 [c]	55.10 ± 2.47[b]	27.07 ± 1.71[c]	112.31 ± 31.37[c]
A. falcatus 600 mg/kg	5.47 ± 1.57[b]	46.26 ± 4.18[c]	60.98 ± 6.52[c]	26.56 ± 1.44[c]	61.55 ± 3.80[c]
B. prionitis 200 mg/kg	6.17 ± 0.37[b]	83.31 ± 14.87	54.70 ± 2.67[a]	25.38 ± 0.97[a]	98.34 ± 35.85[c]
B. prionitis 400 mg/kg	4.91 ± 1.18[c]	59.00 ± 5.88[c]	55.73 ± 3.54[b]	25.83 ± 0.69[b]	92.17 ± 38.79[c]
B. prionitis 600 mg/kg	4.71 ± 1.02[c]	49.06 ± 8.74[c]	59.08 ± 1.27[c]	26.50 ± 1.28[b]	90.94 ± 54.37[c]
Positive control (fosinopril 0.09 mg/kg)	5.50 ± 0.55[c]	60.29 ± 5.64[c]	54.47 ± 2.10[a]	27.83 ± 1.45 [c]	41.99 ± 15.18[c]

Values are expressed as mean ± SD of six animals in each group. Results are significant compared to the ADR induced nephrotoxic control group at: a; $p < 0.05$, b; $p < 0.01$, and c; $p < 0.001$.

of the three selected medicinal plants were greater than 600 mg/kg. The selected doses were found to be safe and therefore they were used in the investigation of nephroprotective activity of the selected medicinal plant extracts.

3.3.2. Nephroprotective activity in ADR induced nephrotoxic rats

3.3.2.1. Change in body weight, consumption of food and intake of water. Effect of selected plant extracts on body weight of nephrotoxic rats is shown in Supplementary file 1, Table 2. A reduction in consumption of food was associated with the induction of nephrotoxicity with ADR. Hence, it could be assumed that the anorexic state of the nephrotoxic rats is responsible for the progressive loss of body weight in experimental animals. There was a significant reduction in the percentage change of body weight in the animals induced with ADR nephrotoxicity compared to the normal control group ($p < 0.05$). Interestingly, the percentage change of body weight was decreased in accordance with the dose related effect in rats treated with *A. moschatus.* This might be due to the loss of weight in plant extracts treated rats (rich in saponin and tannins) as documented in literature (Adeneye and Benebo, 2008).

3.3.2.2. Biochemical analysis. The drug induced nephrotoxicity is associated with a marked elevation in blood urea nitrogen (BUN) and serum creatinine (Adeneye and Benebo, 2008). Elevation of serum creatinine is an indication of the damage to at least a half of the kidney nephrons (Khan et al., 2009, 2010). Hence, increased levels of BUN (43%) and serum creatinine (61%) in the nephrotoxic control group substantiate the nephrotoxicity of ADR in experimental rats. These findings are in agreement with the previous reports of Malarkodi et al. (2003), Yilmaz et al. (2006), El-shitany et al. (2008) and Taskin et al. (2014). Table 3 shows the effect of the treatment with plant extracts on renal biochemical parameters in ADR induced nephrotoxicity.

The treatment with plant extracts showed relatively similar improvement in biochemical parameters in experimental animals as in change in body weight. Treatment at oral doses of 400 and 600 mg/kg/day for three consecutive days lowered the acute elevations in serum concentrations of BUN and creatinine significantly compared to the control rats ($p < 0.05$). However, the direct nephrotoxic effect of ADR, the increased catabolic state of the rats due to prolonged anorexia associated with ADR induced nephrotoxicity might account for the acute elevations in measured biochemical parameters.

Presence of proteinuria is an undoubted sign of renal impairment (Moreira et al., 2017). The glomerular capillary wall is permeable only to the substances with a low molecular weight and low molecular weight proteins are filtered, reabsorbed and metabolized by the proximal tubule cells (Khan et al., 2010). Hence, a high level of proteinuria observed in the present study signifies nephrotoxicity induced by ADR. Damage at the glomeruli and tubules results in an elevation of urinary protein and subsequent reduction in serum protein and albumin in ADR induced nephrotoxic rats. The standard drug, fosinopril was also found to protect the kidney from damage induced by ADR, as evidenced by a decrease in serum creatinine (42%), BUN (38%), proteinuria (86%) and by an increase in serum total protein (12%) and albumin (16%) compared to the nephrotoxic control group. A remarkable reduction of proteinuria might be due to the blockade of the renin– angiotensin system by fosinopril preventing the deterioration of kidney as reported in previous studies (Taskin et al., 2014).

3.3.2.3. Assessment of histopathology. In the present study, induction of nephrotoxicity with a single intraperitoneal dose of ADR produced features of acute tubular necrosis which characterises nephrotoxicity in experimental animals. Accumulation of ADR in the kidney tissues might have caused direct toxic damage to the tubular and glomerular structure of experimental animals leading to acute tubular necrosis and glomerular congestion (Szalay et al., 2015). As shown in Plate 3B, the degenerative tubules showed a loss of tubular brush border, sloughing off of necrotic cells from proximal and distal tubules, tubular vacuolization and pyknosis. Intertubular haemorrhage, glomerular congestion and formation of hyaline casts were the associated features observed. These findings are in agreement with the findings of El-shitany et al. (2008). Table 4 shows the mean scoring values of different groups of animals according to the semi quantitative score system developed by the investigators.

The histopathological findings corroborated the results of biochemical parameters. The concurrent administration of the plant extracts ameliorated ADR induced renal tubular and glomerular alterations in a dose dependent manner as observed in the improved mean histological score values of the groups. The score values for rats treated with plant extracts as well as the standard drug were significantly lower than the values of ADR induced nephrotoxic control group ($p < 0.05$). Intertubular hemorrhage and cytoplasmic vacuolization were the most predominant features observed in ADR induced nephrotoxic rats. Dose dependent decrease in glomerular congestion could be observed in the H and E stained kidney sections of the rats treated with *A. moschatus* (93%, 77%, 70%) and *A. falcatus* (100%, 91%, 85%). Similarly, the rats treated with *A. moschatus* (20%, 15%, 15%) and *B. prionitis* (20%, 8%, 0%) showed a dose dependent decrease in the presence of pyknosis in tubular epithelium. However, loss of brush border (99%) and pyknosis

Table 4

Effect of selected plant extracts on renal histopathology.

Treatment	Score values for the individual features observed						Mean score value
	Loss of brush border	Glomerular congestion	Intertubular haemorrhage	Cytoplasmic vacuolization	Pyknosis	Cast formation	
Healthy control rats	7^{b}	3^{b}	7^{b}	8	1^{b}	0	28^{c}
ADR induced nephrotoxic control rats	9	8	9	9	6	0	44
A. moschatus 200 mg/kg	4	9	10	10	2	0	35
A. moschatus 400 mg/kg	8^{c}	7	9	8	1^{c}	0	35^{b}
A. moschatus 600 mg/kg	3^{c}	7	10	10	1^{a}	0	32^{c}
A. falcatus 200 mg/kg	5^{b}	10	10	10	4	2^{c}	42
A. falcatus 400 mg/kg	6^{b}	9	10	9	0^{c}	0	35^{a}
A. falcatus 600 mg/kg	4^{c}	8	9	10	1^{a}	1^{a}	35^{a}
B. prionitis 200 mg/kg	3	8	10	10	2^{b}	0	34^{b}
B. prionitis 400 mg/kg	7^{b}	3^{b}	8^{b}	8	0^{c}	0	29^{c}
B. prionitis 600 mg/kg	1^{c}	8	10	10	0^{c}	0	30^{c}
Positive control (fosinopril 0.09 mg/kg)	8	6	8^{a}	9	0^{c}	0	34^{b}

Mean score value was given out of the total score of 60. Values are expressed as mean ± SEM of six animals in each group. Results are significant compared to the ADR induced nephrotoxic control group at: a; $p < 0.05$, b; $p < 0.01$, and c; $p < 0.001$.

(69%) were the most distinctive features observed in the control group induced for nephrotoxicity. Those two features were attenuated in the rats treated with plant extracts. The 600 mg/kg dose of *A. moschatus, A. falcatus* and *B. prionitis* extracts showed 70%, 56% and 83% reduction in the loss of brush border and 77%, 81% and 100% reduction in pyknosis respectively. The standard drug, fosinopril showed lesser improvement in the loss of brush border (10%), but a better improvement for the presence of pyknosis (98%) compared to the nephrotoxic control group. However, the rats treated with the standard drug showed better improvement in pyknosis than the rats treated with medicinal plants. Hence, loss of brush border and pyknosis could be mentioned as the most predictive feature of preservation of kidney function based on the findings in the assessment of histopathology. The photomicrographs of the H and E stained kidney sections of the selected groups are shown in Plate 3.

Healthy control group (A), Adriamycin induced nephrotoxic control group (B), Fosinopril treated/positive control group (C), Groups of animals treated with the lyophilized powder of the aqueous extract of *Abelmoschu moschatus* at the three selected doses; 200, 400 and 600 mg/kg (D, E, F), Groups of animals treated with the lyophilized powder of the aqueous extract of *A. sparagus falcatus* at three selected doses; 200, 400 and 600 mg/kg (G, H, I), Groups of animals treated with the lyophilized powder of the aqueous extract of *Barleria prionitis* at three selected doses; 200, 400 and 600 mg/kg (J, K, L) are shown. The features of acute tubular necrosis as shown in Plate 3 (B) are as follow; a: glomerular congestion, b: loss of brush border, c: haemorrhage, d: tubular vacuolization and e: cast formation.

In the present study, treatment with the leaf extracts of *A. moschatus, A. falcatus* and the whole plant extract of *B. prionitis* showed protective effects against ADR-induced nephrotoxicity. It is manifested by the improvements in biochemical parameters and histopathology findings in rats treated with the plant extracts.

According to El-shitany et al. (2008), a number of studies done on animal models of ADR induced nephrotoxicity have demonstrated an increase in the oxidative damage associated with a decrease in the tissue antioxidant status. Hence, products with antioxidant potential are of utmost importance in the mitigation of oxidative damage in the kidney (Dennis and Witting, 2017). Antioxidants mediate detoxification and decomposition of reactive oxygen species, directly involved in the pathogenesis of kidney injury. Therefore, antioxidants with the potency of boosting the endogenous antioxidant activity are considered as potential therapeutic leads in kidney disease (Dennis and Witting, 2017).

Accordingly, several dietary antioxidant compounds including vitamin E, vitamin C and selenium have shown protective effects against

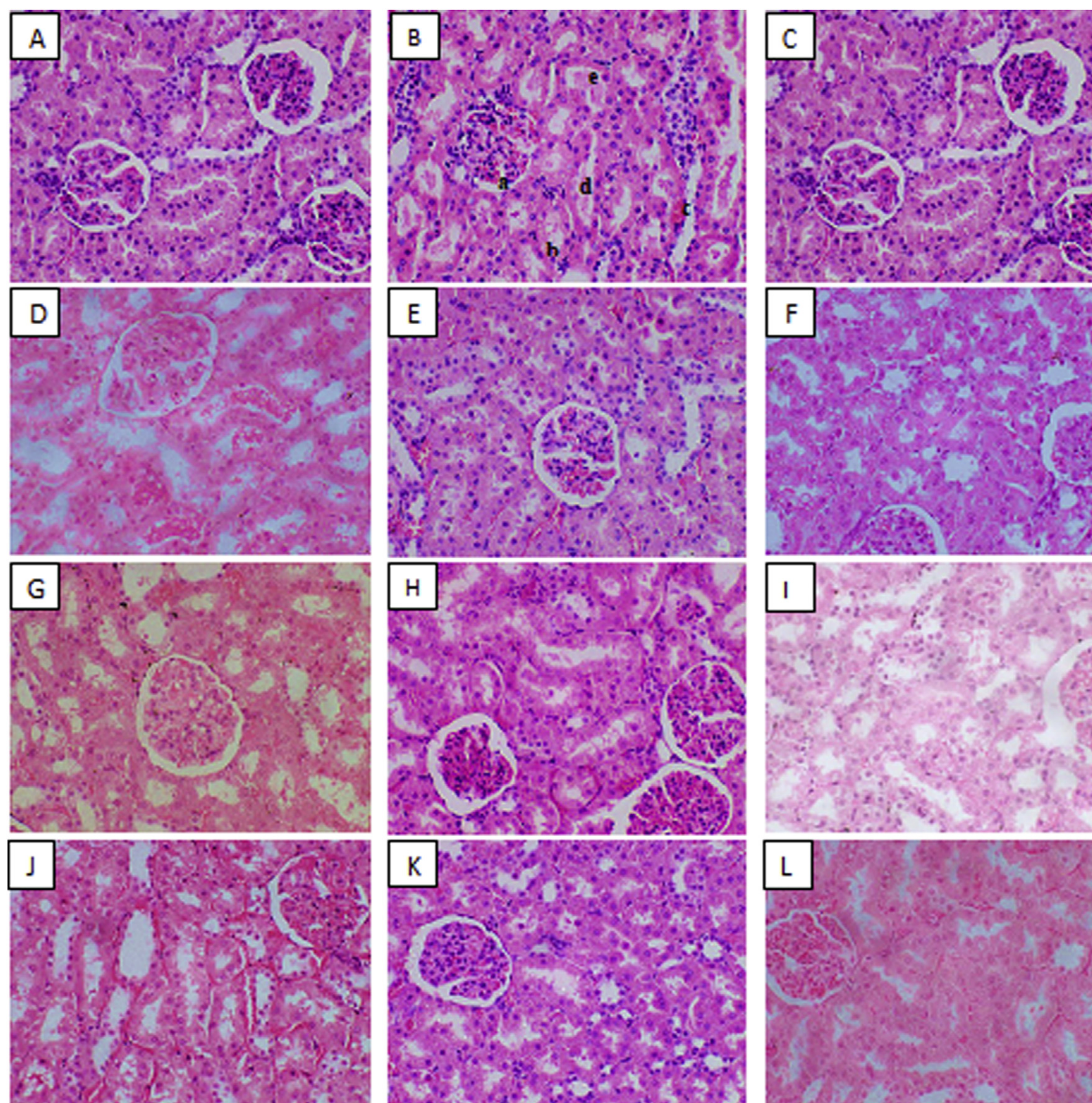

Plate 3. Photomicrographs of kidney tissues of adriamycin induced nephrotoxic rats treated with the aqueous extracts of selected medicinal plants (x400).

oxidative damage attenuating nephrotoxicity. In addition, several dietary plant polyphenols and flavonoids with antioxidant potential including red wine polyphenols, curcumin, quercetin, resveratrol demonstrated protective effects in kidney damage (Dennis and Witting, 2017). These findings substantiate the fact that antioxidant potential arisen from the secondary metabolites of the selected medicinal plants has resulted in protective effects against ADR induced nephrotoxicity.

However, literature support is sparse for the direct benefits of antioxidant supplements on kidney function in patients with acute nephrotoxicity. The reason may be the short intervention period. Persistent changes of kidney function could be achieved generally by long-term treatments (Bolignano et al., 2017). A detailed investigation of the long term nephroprotective effects of the selected medicinal plant extracts in rats with ADR induced nephrotoxicity is in progress in our research laboratory.

4. Conclusions

The standardization of the crude plant material was done based on the physicochemical properties, phytochemicals, TLC and LC-MS profiles. The standardization process would be beneficial in assessing the purity, quality and consistency of the selected plant material. The results of the acute oral toxicity study demonstrated that the aqueous leaf extracts of *A. moschatus*, *A. falcatus* and the whole plant extract of *B. prionitis* at the three selected doses are safe in terms of acute toxic effects. Evaluation of the protective effects based on biochemical parameters and findings in histopathology revealed that the aqueous extracts of the three selected medicinal plants possess significant dose dependent nephroprotective activity against ADR induced acute nephrotoxicity probably mediated through the antioxidant properties. The secondary metabolites present in the plant extracts may attribute to the total antioxidant activities exerting protective effects in free radical pathologies in kidney disease. The long term cellular and molecular nephroprotective mechanisms of the selected plant extracts, isolation and isolation of nephroprotecive compounds are in progress.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jep.2020.112933.

Author Contributions

Conceptualization, APA, KAPWJ, and LKBM; Methodology, SSA; Histopathological analysis, SSA, and LKBM; Validation, APA, LDAMA, KAPWJ, and LKBM; Resources, APA, and LDAMA; Writing the original draft, SSA; Writing, reviewing and editing, all authors; Supervision and guidance in experiments, APA, LDAMA, KAPWJ, and LKBM.

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