



ORIGINAL ARTICLE

Phytochemical evaluation of roots of *Plumbago zeylanica* L. and assessment of its potential as a nephroprotective agent



R. Rajakrishnan^a, R. Lekshmi^{b,*}, P.B. Benil^c, J. Thomas^a, A.H. AlFarhan^a,
V. Rakesh^a, S. Khalaf^a

^a Dept. of Botany and Microbiology, College of Science, King Saud University, P.O. Box 2455, Riyadh, Saudi Arabia

^b Dept. of Botany and Biotechnology, MSM College, Kayamkulam, Kerala, India

^c Dept. of Agadatantra, Vaidyaratnam P.S. Varier Ayurveda College, Edarikode P.O., Kottakkal, Kerala, India

Received 10 November 2016; revised 1 January 2017; accepted 2 January 2017

Available online 8 January 2017

KEYWORDS

Cisplatin;
Histopathology;
Lipid peroxidation;
Nephrotoxicity;
Phytochemical screening

Abstract Search for medicinal plants to treat kidney disorders is an important topic on phytotherapeutic research. *Plumbago zeylanica* L. is an important medicinal plant with hepatoprotective, anti-inflammatory, anti-diabetic, anti-cancer and anti-hyperlipidemic activities. In the present study, the protective effect of hydroalcoholic extract of *P. zeylanica* (HAPZ) in cisplatin induced nephrotoxicity was analyzed in Swiss albino mice. Treatment with higher dose (400 mg/kg) of HAPZ significantly reversed the adverse effect of cisplatin on kidney weight, serum urea and creatinine, indicating their renoprotective effect. The antioxidant effect of the drug is evident from its significant effect on Catalase, Glutathione peroxidase and lipid peroxidation activities.

© 2017 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

1. Introduction

Plumbago zeylanica L. (Plumbaginaceae) is an important medicinal plant greatly valued in Ayurveda for treatment of cough, asthma and gastrointestinal disorders. In *Sushrutha Samhitha* it has been described as antiseptic, febrifuge, detox-

icant, antihelminthic and considered valuable for curing migraine, jaundice, urinary calculi, internal abscesses, seminal weakness, vaginal discharges and insanity. In the Arabian Peninsula, it is mainly distributed over Oman, Yemen and the Southwestern region of Saudi Arabia. The use of the roots of *P. zeylanica* for treating skin problems has been recorded by early Muslim physicians (Ghazanfar, 1994). The hepatoprotective, anti-inflammatory, anti-diabetic, anti-cancer and anti-hyperlipidemic activities of *P. zeylanica* have already been reported (Kanchana and Sadiq, 2011; Thanigavelan et al., 2014; Sunil et al., 2012; Nguyen et al., 2004; Sudha and Sushma, 2009). Cisplatin (cis-diamminedichloroplatinum (II), CDDP) is an important anticancer drug used against the cancers affecting head, neck, lung, testis, ovary, and breast and it

* Corresponding author.

E-mail address: lekshmiradha@gmail.com (R. Lekshmi).

Peer review under responsibility of King Saud University.



Production and hosting by Elsevier

is well known that the main dose-limiting side effect of cisplatin is nephrotoxicity. Many medicinal plants have shown nephroprotective activity and may provide the basis for strategies following the adverse effects of heavy metals, antibiotics, analgesics, anti-cancer drugs and industrial agents. The present study aims to analyze the preliminary phytochemical features of the roots of *P. zeylanica* and also to assess the efficacy of its hydroalcoholic extract (HAPZ) against cisplatin induced nephrotoxicity in Swiss albino mice.

2. Materials and methods

2.1. Plant material

Fresh roots of *P. zeylanica* (Fig. 1) were collected from Adoor, Pathanamthitta district, Kerala and the plant materials were authenticated by Dr. Jacob Thomas, Herbarium, Department of Botany and Microbiology, King Saud University. The roots were cleaned, shade dried, coarsely powdered and stored until further analyses.

2.2. Qualitative phytochemical screening

Weighed quantity of coarsely powdered sample was successively extracted using solvents such as petroleum ether, chloroform, ethyl acetate and methanol. The extracts were concentrated by distillation and excess solvents were removed by evaporation on a water bath. The qualitative phytochemical tests were done according to standard procedures (Raman, 2006).

2.3. GC–MS analysis

One gram of sample was extracted in 100 ml of diethyl ether using Soxhlet apparatus and the extract was concentrated to dryness under vacuum. GC–MS analysis of the diethyl ether extract of the selected drugs was carried out on a 5975C Agilent system equipped with a DB-5 ms Agilent fused silica capillary column (30 × 0.25 mm ID; film thickness: 0.25 µm), operating in electron impact mode at 70 eV. Pure helium (99.9995%) was used as carrier gas at a constant flow of 1.5 mL/min and an injection volume of 1 µL was employed (split ratio is 10:1). Mass transfer line and injector temperature

were set at 230 °C and 250 °C, respectively. The total running time for GC was 35 min. Mass spectra was taken at 70 eV; with a scan range 40–700 m/z. Solvent cut time was 3 min; MS start time being 3 min; MS end time being 35 min; Ion source temperature set to 230 °C and interface temperature being 240 °C. To identify the compounds, the extract was assigned for comparison of their retention indices and mass spectra fragmentation patterns with those stored on the computer library and also with the published literature. National Institute of Standards and Technology library sources (NIST II) were used for matching the identified compounds from the plant materials (McLafferty and Stauffer, 1989).

2.4. Nephroprotective study

2.4.1. Preparation of extract

The roots of *P. zeylanica* were first subjected for purification and detoxification by soaking them in lime water for 48 h and further drying (Shastri, 2012). The shade dried purified drug was pulverized and finely sieved. Weighed quantity of coarse powdered drug was soaked in ethanol (99.9%)/water (1:1) in a percolator for 24 h. The soluble portion was filtered through a filter paper and dried on water bath in a weighed evaporating dish. The extracts were dried under vacuum and stored in desiccator until use for further analysis.

2.4.2. Experimental animals

Swiss albino mice weighing 25 to 30 g body weight were procured from animal house attached to Pharmacology laboratory at SDM Centre for research in Ayurveda and Allied Sciences, Udipi, Karnataka, India. Before the experimental study, approval of Institutional Animal Ethical Committee was taken (SDMCRA/IAEC/RJ18). Animals were housed in 525 × 330 × 230 mm polypropylene cages; 6 mice per cage with paddy husk bedding at temperature 25 °C ± 2 °C and humidity 50 ± 5% during the entire duration of the experimentation. The mice were provided with normal diet and water *ad libitum*.

2.4.3. Experimental design

The animals were grouped into four different categories as mentioned below.



Figure 1 *Plumbago zeylanica* (A) Habit; (B) Dried root; (C) Powdered root.

Group I – Normal control group

Group II – Negative control (Cisplatin 20 mg/kg)

Group III – Test group III (Cisplatin 20 mg/kg + HAPZ 200 mg/kg)

Group IV – Test group IV (Cisplatin 20 mg/kg + HAPZ 400 mg/kg)

Group specific drugs were administered for 10 consecutive days. On 8th day an hour after drug administration a single dose of cisplatin (20 mg/kg body weight) was injected intraperitoneally to all the group except normal control group mice. After 48 h, i.e. on 10th day, an hour after test drug administration the animals were sacrificed and blood was collected from retro-orbital puncture. The blood was allowed to clot and the serum was separated for biochemical estimations. The kidney was dissected out, kept in 10% formalin and used for antioxidant and histological examination.

2.4.4. Assessment of renal function

For the evaluation of renal function, renal parameters such as weight of kidney, serum urea, serum creatinine, serum uric acid, serum sodium and serum potassium were estimated. The blood was collected from the retro-orbital plexus on 10th day from animals and they were anesthetized using sodium phenobarbitone (60 mg/kg). The serum was separated by centrifugation at 1000 rpm for 10 min and analyzed for biochemical parameters such as serum urea, uric acid and electrolytes. Serum urea was measured using the commercially available kit (Liquicheck AGAPPE Diagnostics LTD), following the GLDH-Urease method (Tietz, 1976). The amount of creatinine in serum was estimated using Liquicheck AGAPPE Diagnostics commercial kit according to Picrate method (Cook, 1975). Serum uric acid level was determined using commercially available Biosystems Uric acid kit by Uricase method (Fossati et al., 1980). Estimation of serum sodium and potassium was done by flame photometric method (Chuang et al., 2005).

2.4.5. Estimation of oxidative stress markers

The kidney tissue homogenate was prepared in phosphate buffer saline (pH 7.4) and centrifuged at 4 °C. The clear supernatant was collected and used for estimating catalase activity, glutathione peroxidase activity and lipid peroxidation using standard protocols. Catalase activity in the kidney tissue homogenate was measured according to the procedure of Sinha (1972), determination of glutathione peroxidase was made according to the methodology of Rotruck et al. (1973) and the tissue lipid peroxidation activity was measured following the procedure of Ohkawa et al. (1997).

2.4.6. Statistical analysis

The experimental data were expressed as Mean \pm SEM. Statistical analysis was carried out by one way analysis of variance followed by Dunnet's T₃ multiple comparison test. IBM SPSS Statistics Version 22 was used for the analysis of data.

2.4.7. Histopathological studies

Sections of kidney tissue were histopathologically examined to study the nephroprotective effect of HAPZ. The tissues were fixed in 10% formalin and processed according to a standard protocol (Bancroft and Stevens, 1977). The slides were examined microscopically for pathomorphological changes

such as glomerular congestion, glomerular hypercellularity, interstitial edema, necrosis, tubular casts and hemorrhage.

3. Results

The preliminary phytochemical screening of the roots of *P. zeylanica* showed the presence of sugars, steroids, flavonoids, alkaloids, terpenoids, quinones, phenols, and tannins (Table 1). Alkaloid was seen in ethyl acetate and methanol extracts while terpenoid was noticed only in the chloroform extract.

Gas liquid chromatogram of the diethyl ether extract of root of *P. zeylanica* revealed the presence of 8 peaks indicating the presence of 8 different compounds (Table 2; Fig. 2). Of the 8 constituents, except two all others were matched and identified. The results revealed that 1,4-naphthalenedione (40.09%) was the major component followed by oleic acid (19.95%), β -asarone (14.08%), naphtho (2,3-b)furan-2(3H)-one (7.68%), ethyl *p*-methoxycinnamate (4.58%), and *n*-hexadecanoic acid (2.18%).

3.1. Nephroprotective studies

3.1.1. Kidney weight

There is a significant increase in the weight of kidney by 50.6% in cisplatin administered group in comparison to normal

Table 1 Preliminary phytochemical screening of roots of *P. zeylanica* successive extracts.

Test	Pet ether	Chloroform	Ethyl acetate	Methanol
Alkaloid	–	–	+	+
Carbohydrate	–	+	+	+
Carboxylic acid	–	–	–	–
Coumarins	–	–	–	–
Flavonoids	–	–	–	+
Phenol	–	–	+	–
Quinone	–	+	–	–
Resins	–	–	–	–
Steroid	+	+	+	+
Saponins	–	–	–	–
Tannin	–	–	+	+
Terpenoid	–	+	–	–

Table 2 List of phytochemicals identified by GC–MS of diethyl ether extract of roots of *P. zeylanica*.

Peak	RT	% Area	Name	Match
1	13.050	14.08	β -Asarone	Identified
2	13.225	40.09	1,4-Naphthalenedione	Identified
3	13.657	2.31	–	Unidentified
4	13.857	4.58	Ethyl <i>p</i> -methoxycinnamate	Identified
5	14.720	2.18	<i>n</i> -hexadecanoic acid	Identified
6	14.764	9.13	–	Unidentified
7	14.945	7.68	Naphtho(2,3-b)furan-2(3H)-one	Identified
8	15.546	19.95	Oleic Acid	Identified

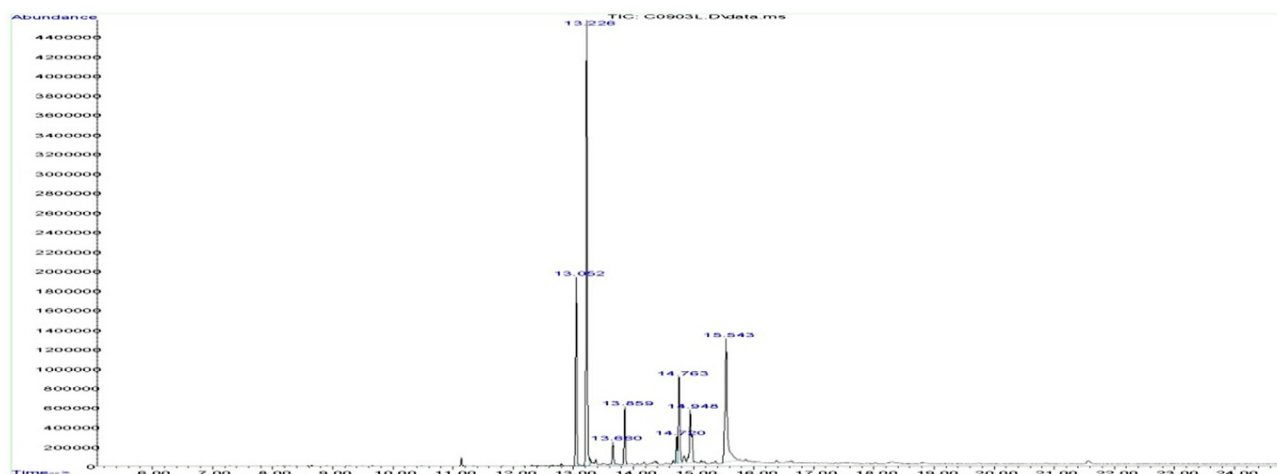


Figure 2 GC-MS of diethyl ether extract of roots of *P. zeylanica*.

control group. HAPZ administered at higher dose level has shown significant decrease in kidney weight when compared to cisplatin control group but the lower dose of HAPZ has no effect in this aspect.

3.1.2. Serum urea

In cisplatin administered group there was a remarkable significant increase (228.67%) in the serum urea level in comparison to the normal control group. The results indicated that the drug showed a dose dependent significant reduction in the serum urea level toward normal range. The higher dose of HAPZ reduced the concentration of urea by 68.2% when compared to cisplatin control group.

3.1.3. Serum creatinine

In cisplatin administered group there was a significant increase in the serum creatinine level (3.0 ± 0.19 mg/dl) in comparison to normal control group (0.7 ± 0.05 mg/dl). HAPZ exhibited a dose dependent significant decrease in the serum creatinine level. The creatinine level was reduced to 0.85 ± 0.06 mg/dl (71.67%) by the administration of higher dose (400 mg/kg) of HAPZ.

3.1.4. Serum uric acid

The concentration of serum uric acid was increased significantly by 81.5% in cisplatin control group when compared to normal control group. HAPZ administered group exhibited increase in the serum uric acid level in comparison to cisplatin control group, but was statistically insignificant.

3.1.5. Serum sodium and potassium

There was only a small insignificant increase in serum sodium level in the cisplatin administered group in comparison to normal control group. HAPZ administered at both dose levels has shown decrease in the serum sodium level in comparison to cisplatin control group and the effect of the higher dose was found to be statistically significant.

Serum potassium level was significantly decreased by 32.43% in the cisplatin administered group in comparison to normal control group. HAPZ exhibited dose dependent reduction but the effect of higher dose alone was statistically significant. The results are represented in [Table 3](#).

3.1.6. Effect of HAPZ on kidney tissue oxidative stress markers

To investigate the antioxidant potential HAPZ and its impact on cisplatin induced nephrotoxicity, oxidative stress enzyme markers such as catalase and glutathione peroxidase as well as lipid peroxidation were estimated ([Table 4](#)). The mean concentration of catalase (CAT) in the kidney tissue of normal control mice was 98.34 ± 1.37 which was significantly reduced to 16.63 ± 2.48 in cisplatin treated mice. Both doses of HAPZ showed significant increase in the activity of catalase but the lower dose exhibited pronounced effect than that of the higher dose of HAPZ. Glutathione peroxidase (GHX-px) concentration in the kidney tissue of cisplatin control mice was 3.08 ± 0.63 $\mu\text{mol}/\text{mg}$ protein, whereas in normal control mice 12.04 ± 0.65 $\mu\text{mol}/\text{mg}$ protein, which indicate a significant reduction in glutathione peroxidase activity in nephrotoxic animals. Treatment with higher dose (400 mg/kg) of HAPZ

Table 3 Effects of HAPZ on Kidney weight, Serum urea, Serum creatinine, Serum uric acid, Serum sodium and potassium.

Groups	Kidney weight (mg)	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)	Sodium (mmol/L)	Potassium (mmol/L)
Normal Control	318.00 \pm 7.92	40.67 \pm 1.58	0.7 \pm 0.05	2 \pm 0.13	146.00 \pm 2.26	5.18 \pm 0.26
Cisplatin control	479.00 \pm 15.68 ^{###}	133.67 \pm 2.06 ^{###}	3.0 \pm 0.19 ^{###}	3.63 \pm 0.23 [#]	148.5 \pm 1.04	3.50 \pm 0.24 [#]
HAPZ 200	465.71 \pm 24.66	42.50 \pm 1.94 ^{***}	0.85 \pm 0.06 ^{**}	2.48 \pm 0.40	144.00 \pm 1.09	5.00 \pm 0.36
HAPZ 400	375.00 \pm 13.38 ^{**}	47.17 \pm 2.98 ^{***}	1.08 \pm 0.10 ^{**}	2.78 \pm 0.18	141.00 \pm 0.88 [*]	5.23 \pm 0.19 [*]

Data: MEAN \pm SEM, ^{###}P < 0.001 in comparison to normal control group, ^{##}P < 0.01 in comparison to normal control group, [#]P < 0.05 in comparison to cisplatin control group, ^{***}P < 0.001 in comparison to cisplatin control group, ^{**}P < 0.01 in comparison to cisplatin control group, ^{*}P < 0.05 in comparison to cisplatin control group.

Table 4 Effect of HASA and HAPZ on antioxidant parameters.

Groups	Catalase (μM of H_2O_2 consumed/min/mg protein)	Glutathione peroxidase (μM of GSH oxidized/min/mg protein)	Lipid peroxidation (μM of MDA formed/g wet tissue)
Group I	98.34 \pm 1.37	12.04 \pm 0.65	26.98 \pm 3.05
Group II	16.63 \pm 2.48 ^{###}	3.08 \pm 0.63 ^{##}	160.99 \pm 8.78 ^{###}
Group III	86.39 \pm 2.93 ^{**}	8.76 \pm 0.97 ^{**}	39.95 \pm 7.92 ^{***}
Group IV	77.95 \pm 1.91 ^{**}	13.60 \pm 0.54 ^{**}	45.33 \pm 8.92 ^{**}

Data: MEAN \pm SEM, ^{###}P < 0.001 in comparison to normal control group, ^{##}P < 0.01 in comparison to normal control group, ^{***}P < 0.001 in comparison to cisplatin control group, ^{**}P < 0.01 in comparison to cisplatin control group.

significantly increased the level of glutathione peroxidase toward the normal level. The lower dose of HAPZ also showed significant activity. Malondialdehyde (MDA) levels in kidney tissue, used as an index of lipid peroxidation was significantly higher (160.99 \pm 8.78) in cisplatin treated group when compared to normal control group (26.98 \pm 3.05). HAPZ exhibited significant effect on decreasing the MDA concentration in cisplatin treated animals. HAPZ at 200 mg/kg dose reduced MDA level by 75.18% while the higher dose of 400 mg/kg decreased MDA level by 71.84%.

3.1.7. Histopathological examination of kidney tissue

Microscopic examination of the kidney sections from normal control group mice exhibited normal intact cytoarchitecture in both cortex and medulla. Glomeruli were numerous and well developed. Convoluted tubules and their epithelial lining were normal (Fig. 3A). Microscopic examination of sections of kidney from cisplatin only injected control group showed significant degenerative changes in both cortex and medulla. Cellularity of the glomeruli was decreased as well as epithelial lining of convoluted tubules showed erosion and necrotic

changes. Edematous changes were visible in the interstitial tissue, focal cell infiltration was observed at certain places, hemorrhage and degenerative changes were detected in the medulla (Fig. 3B). Microscopic examination of sections of kidney from cisplatin injected and HAPZ 200 mg/kg dose receiving mice exhibited mixed profile. Very good protection was observed in 3 mice and weak to moderate protection was observed in the remaining. In those mice showing good protection, the cytoarchitecture looked almost normal with only few degenerative changes in the epithelial lining. In the remaining, moderate degenerative changes in the tubular epithelium and interstitial tissue edema were observed (Fig. 3C). Microscopic examination of sections of kidney from cisplatin injected and HAPZ 400 mg/kg dose receiving mice also showed almost similar microscopic profile to HAPZ 200 mg/kg dose receiving mice except absence of interstitial edema (Fig. 3D).

4. Discussion

Qualitative phytochemical screening of herbal drugs is a prerequisite study prior to their detailed phytochemical and

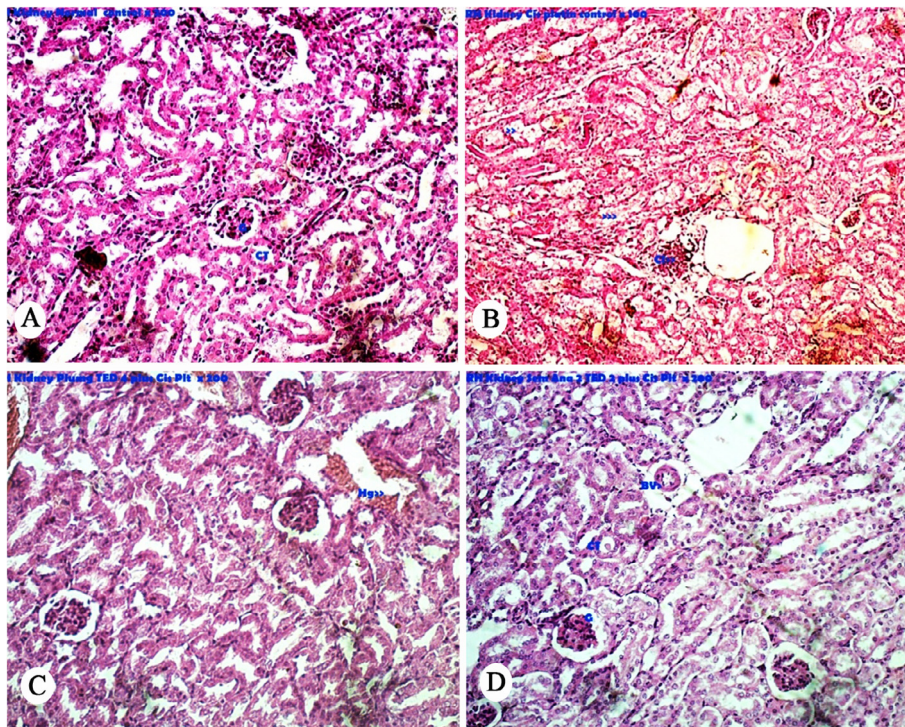


Figure 3 Histopathological examinations in the kidney tissue sections of mice of different groups (A) Normal control; (B) Cisplatin control; (C) HAPZ 200 mg/kg treated and (D) HAPZ 400 mg/kg treated.

pharmacological investigation. Various tests have been conducted for the qualitative determination of bioactive compounds in the roots of *P. zeylanica*. In the present investigation, qualitative analysis of four different extracts (petroleum ether, chloroform, ethyl acetate and methanol) of the drug was analyzed for phytochemicals. Different solvents have various degrees of solubility for different phytochemicals (Majorie, 1999). Sugars, steroids, flavonoids, quinones, phenols, tannins and terpenoids were present in the roots of *P. zeylanica*. The present study demonstrated that cisplatin induced renal injury is evident from the increased kidney weight, high values of creatinine, urea and uric acid in serum, elevated concentration of malondialdehyde (MDA) and decreased level of catalase and glutathione peroxidase in kidney tissue of the experimental animals (Swiss albino mice). Administration of HAPZ ameliorates the nephrotoxicity induced by cisplatin in animals. It is reported that in cisplatin induced nephrotoxicity, kidneys gain weight as the damage increases (Ravindra et al., 2010). In agreement with the other reports, the present study also revealed that there is significant increase in kidney weight in cisplatin treated experimental organisms. Increase in the weight of the kidney following cisplatin administration is probably due to the renal cell injury caused by inflammation. When there is inflammation of an organ or tissue, blood flow to the area increases and there is a transient stasis of blood in the area of inflammation, permitting inflammatory cells like neutrophils and monocytes along with fluid to extravagate from the vascular compartment to the interstitium which probably leads to increase in the weight of kidney. The higher doses of HAPZ significantly decreased the kidney weight in treated animals. The reduction in kidney weight after the administration of HAPZ can be attributed to their anti-inflammatory potential.

The serum urea, creatinine and uric acid are the ultimate metabolites of purine which may alter the glomerular filtration rate and increase in their levels in serum are associated with renal damage and considered as the index of nephrotoxicity (Mika and Guruvayoorappan, 2013). Serum creatinine concentration is believed to be a more potent indicator than the urea and uric acid levels in the first phase of any kidney disease (Tavafi et al., 2012). It is reported that urea concentrations in serum begin to increase only after parenchymal injury (Gilbert et al., 1989). Brenner and Hostetter (1987) reported that elevation in the concentration of uric acid may also lead to progressive renal insufficiency. The present study clearly revealed that administration of HAPZ successfully prevented cisplatin induced renal damage in mice by significantly reducing the elevated levels of urea, creatinine and uric acid in serum. Cisplatin administration has produced considerable damage to nephrons as indicated by the significant elevation of serum urea concentration. HAPZ at both doses offered significant renal protection by reducing the serum urea. Cisplatin administration also elevated serum creatinine level significantly indicating its nephrotoxicity probably by damaging the renal parenchymal cells. HAPZ at both doses significantly reduced serum creatinine and proclaimed its nephroprotective behavior by reversing the cellular parenchyma damage induced by cisplatin. Hyperuricemia, increase in uric acid level is associated with renal disease, but it is usually considered a marker of renal dysfunction rather than a risk factor for progression (Amin-ul-Haq et al., 2010). HAPZ at both doses have exhibited an insignificant effect on uric acid reduction.

Higher doses of drugs, toxins, infectious agents, chemicals etc. can cause kidney damage and ultimately lead to electrolyte imbalance (Shimmi et al., 2012). The concentration of serum sodium did not show a significant change (only less than 2% increase) in cisplatin treated group when compared to the normal group. Similar results, however, have been reported with respect to changes in serum sodium after cisplatin treatment (Vijayan et al., 2007). The present study revealed a significant decrease in potassium level after the administration of cisplatin. It is previously reported that hypokalemia, a common electrolyte abnormality occurred during cisplatin treatment due to increased renal reabsorption capacity observed in response to decreased intestinal absorption of potassium (Arunkumar et al., 2012). The administration of higher doses of HAPZ significantly increased the potassium concentration toward normal values in cisplatin treated groups, which indicates its potential to overcome potassium imbalance.

The role of oxidative stress in cisplatin-induced nephrotoxicity is also reported by many researchers. Studies have demonstrated that cisplatin induces the formation of radical oxygen species in renal epithelial cells primarily by decreasing the activity of antioxidant enzymes (Ozbek, 2013). It was reported that oxidative stress upon cisplatin administration is characterized by increased lipid peroxidation and altered non-enzymatic and enzymatic antioxidant systems (Hawkins et al., 2001). In this study, oxidative stress induced by the administration of cisplatin is evidenced by the reduced catalase and glutathione peroxidase levels as well as increased lipid peroxidation.

The reduction in the activity of catalase in the cisplatin treated group results in decreased ability to scavenge toxic hydrogen peroxide, further contributing to oxidative stress HAPZ exhibited statistically significant elevation in catalase level; however the lower dose was somewhat more effective than its higher dose. The activity of glutathione peroxidase enzyme also got decreased following cisplatin administration. HAPZ showed dose related increase in the glutathione peroxidase concentration when compared to the normal control group. Administration of HAPZ normalize the activities of catalase and glutathione peroxidase enzymes which might be due to the presence of free radical scavenging activity of flavonoids present in these drugs (Nile and Khobragade, 2010). The membrane lipid peroxidation, the primary event in the tissue damage, which was found to be elevated in cisplatin control group was significantly reduced and brought toward normal by the administration of HAPZ. This shows the capability of this drug to reverse the membrane damages induced by cisplatin. The results clearly indicated the antioxidant potential of HAPZ but its effect on catalase activity and lipid peroxidation was not dose dependent. It can be suggested that the HAPZ contains many active principles, some of which may have mutually antagonistic effects and because of this, at higher dose level the effect of the antioxidant active principle may get reduced.

It was reported that the toxic effect of cisplatin in kidney can be clearly explained by observing the cytotoxic injury which ranges from mild sub-lethal variations to necrotic death. (Dobyn et al., 1980). Histopathological studies in mice kidney after cisplatin administration revealed acute tubular necrosis which confirms irreversible injury to kidney (Pratibha et al., 2010). In the present study, the histopathology of kidney sections from cisplatin control group revealed necrotic changes in the tubular epithelium, edematous changes in the interstitial

tissue and focal cell infiltration. Microscopic examination of sections of kidney from cisplatin injected and HAPZ at 200 mg/kg as well as 400 mg/kg dose treated mice revealed that the drug at both doses provide moderate to good protection as the cytoarchitecture looked almost normal with only few degenerative changes in the epithelial lining and tubular epithelium.

5. Conclusion

In this study, roots of *Plumbago zeylanica* were subjected for phytochemical examination as well as its potential as a nephroprotective agent. Preliminary phytochemical analysis revealed the presence of sugars, steroids, flavonoids, alkaloids, quinones, phenols, tannins and terpenoids in its roots. Gas liquid chromatogram of the diethyl ether fraction of the roots of *P. zeylanica* revealed the presence of 8 peaks of which 6 were identified. The hydroalcoholic extract of the drug was found to produce significant reversal of cisplatin induced changes in the kidney as indicated by measured biochemical parameters at higher dose of 400 mg/kg b.w.

Acknowledgements

The authors would like to extend their sincere appreciation to the Deanship of Scientific Research at King Saud University, Saudi Arabia for funding this Research (RG-1437-002). Authors are highly grateful to Dr. B. Ravishankar, Director, SDM Centre for Research in Ayurveda & Allied Sciences, Udupi, Karnataka, India for providing facility to carry out pharmacological studies.

References

- Amin-ul-Haq, Mahmood, R., Ahmad, Z., Jamil-ur-Rehman, Jilani, G., 2010. Association of serum uric acid with blood urea and serum creatinine. *Pak. J. Physiol.* 6 (2), 46–49.
- Arunkumar, P.A., Viswanatha, G.L., Radheshyam, N., Mukund, H., Belliyappa, M.S., 2012. Science behind cisplatin induced nephrotoxicity in humans: A clinical study. *Asian Pac. J. Trop. Biomed.* 2 (8), 640–644.
- Bancroft, J.D., Stevens, A., 1977. Theory and practice of histological techniques. *J. Am. Med. Assoc.* 238, 2730.
- Brenner, B.M., Hostetter, T.H., 1987. Tubulointerstitial diseases of kidney. In: Harrison's Principles of Internal Medicine. 11th ed. McGraw Hill Book Company, New York, pp. 1150–1156. Vol. 2.
- Chuang, F.S., Sarbeck, J.R., Winefordner, J.D., 2005. Flame spectrometric determination of sodium, potassium and calcium in blood serum by measurement of microsamples. *Clin. Chem.* 21, 16–23.
- Cook, J.G., 1975. Factors influencing the assay of creatinine. *Ann. Clin. Biochem.* 12 (6), 219–232.
- Dobyn, D.C., Levi, J., Jacobs, C., Kosek, J., Weiner, M.W., 1980. Mechanism of cisplatin nephrotoxicity: morphologic observations. *J. Pharmacol. Exp. Ther.* 213, 551–556.
- Fossati, P., Prencipe, L., Berti, G., 1980. Use of 3,4-dichloro-2-hydroxybenzenesulfonic acid/ 4 aminophenazone chromogenic system in direct enzymic assay of uric acid in serum and urine. *Clin. Chem.* 26 (2), 227–231.
- Ghazanfar, S.A., 1994. Handbook of Arabian Medicinal Plants. CRC Press, Florida, pp. 168–169.
- Gilbert, D.N., Wood, C.A., Kohlhepp, S.J., Kohnen, P.W., Houghton, D.C., Finkbeiner, H.C., Lindsley, J., Bennett, W.M., 1989. Polyaspartic acid prevents experimental aminoglycoside nephrotoxicity. *J. Infect. Dis.* 159, 945–953.
- Hawkins, C.L., Brown, B.E., Davies, M.J., 2001. Hypochlorite and hypobromite mediated radical formation and its role in cell lysis. *Arch. Biochem. Biophys.* 395, 137–145.
- Kanchana, N., Sadiq, M., 2011. Hepatoprotective effect of *Plumbago zeylanica* on paracetamol induced liver toxicity in rats. *Int. J. Pharm. Pharma. Sci.* 3 (1), 151–154.
- Majorie, M.C., 1999. Plant products as antimicrobial agents. *Clin. Microbiol. Rev.* 12 (4), 564–582.
- McLafferty, F.W., Stauffer, D.B., 1989. Wiley/NBS Registry of Mass Spectral Data. Wiley, New York.
- Mika, D., Guruvayoorappan, C., 2013. The effect of *Thespesia populnea* on cisplatin induced nephrotoxicity. *J. Can. Res. Ther.* 9, 50–53.
- Nguyen, A.T., Malonne, H., Duez, P., Faotre, R.V., Vanhaelen, M., Fontaine, J., 2004. Cytotoxic constituents from *Plumbago zeylanica*. *Fitoterapia* 75 (5), 500–504.
- Nile, S.H., Khobragade, C.N., 2010. Antioxidant activity and flavonoid derivatives of *Plumbago zeylanica*. *J. Nat. Prod.* 3, 130–133.
- Ohkawa, H., Ohishi, N., Yagi, K., 1997. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* 95, 351–358.
- Ozbek, E., 2013. Induction of oxidative stress in kidney. *Int. J. Nephrol.* <http://dx.doi.org/10.1155/2012/465897>.
- Pratibha, R., Dayanand, A.B., Sameer, K., Padmanabh, V.R., Chitra, Y.D., 2010. Cisplatin induced histological changes in renal tissue of rat. *J. Cell Anim. Biol.* 4 (7), 108–111.
- Raman, N., 2006. Phytochemical Techniques. New Indian Publishing Agencies, New Delhi, pp. 19–32.
- Ravindra, D.A., Kulkarni, S.S., Padmanabha, V., Chitra, Y., Dhume, K.U., 2010. Cisplatin induced histological changes in renal tissue of rat. *J. Cell. Anim. Biol.* 4, 108–111.
- Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G., Hoekstra, W.G., 1973. Selenium: biochemical role as a component of glutathione peroxidase. *Science* 179, 588–590.
- Shastri, K., 2012. Sadananda Sharma: Rasa Tarangini. Motilal Banarasidas, New Delhi, pp. 651–652.
- Shimmi, S.C., Jahan, N., Sultana, N., 2012. Effects of ashwagandha (*Withania somnifera*) root extract against gentamicin induced changes of serum electrolytes in rats. *J. Bangladesh Soc. Physiol.* 7 (1), 29–35.
- Sinha, K.A., 1972. Colorimetric assay of catalase. *Anal. Biochem.* 47, 389–394.
- Sudha, R.P., Sushma, A.M., 2009. Antihyperlipidemic effect of aqueous extract of *Plumbago zeylanica* roots in diet-induced hyperlipidemic rat. *Pharm. Biol.* 47 (10), 1004–1010.
- Sunil, C., Duraipandiyar, V., Agastian, P., Ignacimuthu, S., 2012. Antidiabetic effect of plumbagin isolated from *Plumbago zeylanica* L. root and its effect on GLUT4 translocation in streptozotocin induced diabetic rats. *Food Chem. Toxicol.* 50, 435–446.
- Tavafi, M., Ahmadvand, H., Toolabi, P., 2012. Inhibitory effect of olive leaf extract on gentamicin-induced nephrotoxicity in rats. *Iranian J. Kidney Dis.* 6 (1), 25–32.
- Thanigavelan, V., Venkatachalam, K., Venkatachalam, L., Natarajan, S., Murugan, P.K., Savarimuthu, J.A., 2014. Hydroalcoholic extract of *Plumbago zeylanica* Linn root bark exhibit analgesic and anti-inflammatory activities in experimental rat models. *Am. J. Pharm. Health Res.* 2 (4), 209–221.
- Tietz, N.W., 1976. Textbook of Clinical Chemistry. Saunders, Philadelphia W.B.
- Vijayan, F.P., Rani, V.K., Vineesh, V.R., Sudha, K.S., Michael, M. M., Padikkala, J., 2007. Protective effect of *Cyclea peltata* Lam on cisplatin-induced nephrotoxicity and oxidative damage. *J. Basic Clin. Physiol. Pharmacol.* 18 (2), 101–114.