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STUDIES ON NEPHROPROTECTIVE AND NEPHROCURATIVE ACTIVITY OF ETHANOLIC EXTRACT OF PICRORHIZA KURROA ROYLE AND AROGYAWARDHINI BATI IN RATS

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ABSTRACT:

Aim: To evaluate nephroprotective and nephrocurative activity of rhizome of *Picrorhiza kurroa*.

Materials and Methods: The ethanolic extract of rhizome of *P. kurroa* was studied for nephroprotective and nephrocurative effect in female Wistar rats against Cisplatin (5mg/kg b.w.i.p.) induced nephrotoxicity, by estimating serum creatinine and blood urea levels. One of the Ayurvedic formulations viz. Arogyawardhini, containing *P. kurroa* as a major ingredient was also studied for the nephroprotective and nephrocurative effects against Cisplatin induced nephrotoxicity. The formulation was standardized for the presence of total polyphenols.

Results: Treatments with the ethanolic extract of the rhizome in the dose of 600 mg/kg b.w.p.o.could significantly ($P < 0.001$) reduce the elevated serum levels of creatinine and blood urea.

Conclusion: The formulation was found to have better activity as compared to the rhizome.

Keywords: *Picrorhiza kurroa* · Cisplatin · Nephrotoxicity · Nephroprotection Antioxidant**.**

INTRODUCTION

The incidence of kidney failure or chronic kidney failure has doubled over the last 15 years. It is estimated that currently, there are over one million people worldwide who are alive on dialysis or with a functioning graft. Diabetes is an important cause of kidney failure and diabetes is five times more common in the Asians when compared to the white population. Another life style related disorder viz. Asians again are twice more prone to develop this condition in comparison to the white population. Almost 66% kidney failure occurs due to hypertension or diabetes. There are approximately 7.85 million people suffering from chronic kidney failure in India. In India 90% patients who suffer from kidney disease are not able to afford the cost of treatment. The crisis of kidney shortage is a global phenomenon and it is worst in Asian countries [1]. Renal fibrosis is a common consequence of progressive renal diseases. In nearly all cases, the extent of fibrotic lesions strongly correlates with disease severity and eventual progression to end-stage renal disease (ESRD) [2]. Modern day therapy of renal disease includes dietary protein restriction, blood pressure control, angiotensin-converting enzyme inhibitors (ACEIs), and angiotensin receptor blockers (ARBs) [3]. Kidney being one of the vital organs of human body performing the function of detoxification needs protection for healthy life. As stated above, the prevalence of the kidney disorders needs to be seriously viewed. Many herbal drugs have been investigated for various ailments, however, very few herbs have been considered for nephroprotection. The herbal drugs used and studied for their utility in protection of kidney include *Astragalus* and a mixture of *Astragalus, Angelica, Ligusticum, Triptolide* and *Rhubarb*, have a beneficial role in slowing the progression of renal failure. This effect is multifunctional and multi-targeted; moreover, it is often associated with a reduction in proteinuria and the amelioration of dyslipidaemia, but not with changes in systemic blood pressure [4].

The roots of *Picrorrhiza kurroa* are utilized clinically in Ayurveda for treatment of hypertension and cardiac disorders. It is also well known drug for treatment of liver disorders. The drug has not yet explored

for the treatment of kidney disorders and there is a high probability that the drug would prove to be beneficial in offering protection against the kidney failure in patients suffering from diabetes, hypertension and cardiac disorders.

Natural products have been utilized as an important resource for the maintenance of life for ages. Already in the earliest written traditions, *e.g.* the Rig-Veda of South Asia *ca.*1500-900 BC), it is evident that plants played an important role in daily life. One of the Species that emerged from such an inventory is *Picrorhiza kurroa* Royles [5].The dried rhizome of *P. kurroa*, (*Scrophulariaceae*), a small plant indigenous to the Alpine Himalayas, contains at least 60% of a 1:1.5 mixture of Picroside-I and kutkoside and the remainder (40%) is a mixture of iridoid as well as cucurbitacin glycosides [6].The drug is well evaluated as a hepatoprotective one and acts probably by scavenging the free radicals and inhibition of generation of oxygen species.

The roots have found to lower the blood glucose levels of alloxan induced diabetes in rats [7].With this background the study has been designed with the aim to evaluate the roots of *P.kurroa (Scrophulariaceae)* for its nephroprotective activity so that the drug can be utilized to protect kidneys, as the organ needs serious attention in the modern life style.

2. MATERIALS AND METHODS

2.1 Animals

Healthy adult female Wistar rats (150-250gm each) aged 60-90 days were used for the study. The rats were housed in polypropylene cages and maintained under standard condition (12hrs light/dark cycle; 25±30C; 35-60% RH). Animals were fed with commercially available rat and mice feed (Nav Maharashtra Chaken Oil Mills Ltd., Pune, India) and tap water provided *ad libitum*. The protocols of the study were approved by Institutional Animal Ethical Committee (IAEC).

2.2 Plant Material

The dried rhizomes of *P. kurroa* were procured from local market, Mumbai. The drug was authenticated at the Agharkar Research Institute, Pune India. A Voucher specimen (AHMA R 095) has been deposited in Agharkar Research Institute, Pune India. Arogyawardhini bati manufactured by Baidyanath (batch no. 080612) was utilized for the present work.

2.3 Drugs and chemicals

The following chemicals were used for the study, Cisplatin injection (Cipla, Ltd. Batch no.J80340, Mumbai, India), Urea estimation kit (Precilab Reagent and chemicals Pvt.Ltd, Navi-Mumbai), Creatinine estimation kit (Coral Clinical System, Goa, India), Arogyawardhini bati manufactured by Baidyanath (batch no. 080612).

2.4 Preparation of the extracts

The shade dried, powdered rhizomes (250gm) of *P. kurroa* were defatted by extracting with petroleum ether (60-80^{0}C), followed by extraction with ethanol using Soxhlet extractor. The ethanolic extract was then concentrated rotary flash evaporator to a syrupy consistency. The residual solvent was removed by drying the extract in vacuum oven (yield - 25.5gm).

Powdered Arogyawardhini bati (25gm) was defatted by extracting with petroleum ether (60-80^{o}C) and then with ethanol using Soxhlet extractor. The ethanolic extract was then concentrated in vacuum (yield 0.87 gm) and was utilized for phytochemical analysis.

2.5 Phytochemical analysis

The ethanolic extracts of *P. kurroa* and Arogyawardhini bati were tested qualitatively for presence of different phytoconstituents using various chemical tests [12]. The content of the total polyphenols in the ethanolic extract of Arogyawardhini bati and *P. kurroa* extract was determined using Folin-Ciocalteu method [13].using Gallic acid (Molychem, India) as a reference standard.

2.6 Acute toxicity studies

Acute oral toxicity studies were performed as per OECD Guidelines 425. Dosed one animal at the dose of 3000mg/kg b.w.p.o. The animal was observed continuously for 2hrs for gross behavioral changes and the intermittently once every 2hrs and finally at 24 and 72hrs to note any signs of toxicity including death.

2.7 Experimental procedure

Cisplatin- induced renal injury

Three major groups of six rats each were used in this model. Control group was administered with 2% w/v gum acacia for 6 and 16 days. On the 7^{th} and 17^{th} day blood was withdrawn by puncturing retro-orbital plexus from the animals of control group. The extent of renal damage was determined by treating toxicant groups with a single dose of Cisplatin 5mg/kg b.w.i.p. [14].

On the 7^{th} and 17^{th} day blood was withdrawn through retro-orbital plexus from the animals of toxicant groups.

2.8 Evaluation of Nephroprotective activity

The animals were divided into three major groups viz. control, toxicant and test with six animals in each group. The control group received vehicle (gum acacia mucilage 2% w/v) once a day for 6 days.

The toxicant group was administered single dose of Cisplatin (5mg/kg b.w.i.p. Cipla, Ltd. Batch no.J80340) on day 1. The test groups were treated with different doses of *P. kurroa* and Arogyawardhini extracts (300 and 600mg/kg b.w.p.o) once a day, from day 1, for 6 days along with single dose of Cisplatin (5mg/kgb.w.i.p) on day 1. In all the groups blood Samples were withdrawn on day 7 by puncturing retro-orbital plexus for estimation of biochemical parameters [15].

2.9 Evaluation of Nephrocurative activity

The animals were divided into three major groups' viz. control, test, and toxicant with six animals in each group. The control group received vehicle (gum acacia mucilage 2% w/v) once a day for 16 days. The toxicant group was administered single dose of Cisplatin (5mg/kg b.w.i.p) on day 1. The test groups were treated with different doses of *P .kurroa* and Arogyawardhini extracts (300and 600mg/kg b.w.p.o) once a day, from day 6 onwards till day 16. In all the groups blood samples were withdrawn on day 17 by puncturing retro orbital plexus for estimation of biochemical parameters [15].

2.10 Assessment of renal function

The blood samples were allowed to clot for 30 min. The serum was separated by centrifuging the blood clots and further utilized for estimation of biochemical parameters like urea and creatinine levels.

Blood urea levels were estimated by enzymatic method using Urease enzyme kits [16].Absorbance was read at 578nm on UV- Vis spectrophotometer (Elico 159).Serum creatinine levels were estimated by alkaline picrate method using creatinine kit [16]. Absorbance was read at 490nm on UV- Vis spectrophotometer (Elico 159). The weight of the animals was monitored at various stages viz on day 1, day 7 (preventive regimen), day 17 (curative regimen), before and after the treatments.

2.11 Histopathological examination

Two animals from each group were sacrificed on the day of blood withdrawal. The kidneys were isolated and fixed in 10% neutral formalin solution [17]. Both the kidneys were processed further for embedding in paraffin wax to take the section. The sections were stained with hematoxylin and eosin and observed under light microscopy.

2.12 Statistical analysis

The data obtained from the animals studies were analyzed using one-way ANOVA followed by Bonferroni Multiple Comparisons Test using Graph pad in stat (Version 3.05).

2.13 In vitro Antioxidant study

Antioxidant studies were carried out by the nitric oxide scavenging method [8]. In this assay, 0.3 ml of Sodium nitroprusside (5mM) in phosphate buffered saline was mixed with 1ml of the aqueous solutions of the extracts, having concentrations in the range of 50 to 500µg/ml. The assay mixture was then treated with Griess' reagent and the optical density of the resultant chromophore was determined spectrophotometrically at 546nm. The absorbance values were compared with the absorbance values of the standard solution of ascorbic acid simultaneously run in identical assay units (fig 7-9). The experiment was carried out in triplicate.

3. RESULTS

Quantitative phytochemical analysis of *P. kurroa and* Arogyawardhini Bati revealed presence of tannins 0.004468 and 0.049% w/w in the ethanolic extracts respectively. The ethanolic extract of *P. kurroa,* at the dose of 3000mg/kg b. w. p. o., did not produce any mortality and any significant changes in the autonomic or behavioral responses in the 48 hours later the administration of the dose. Therefore, the root can be considered to be practically safe.

Animal model of Cisplatin induced nephrotoxicity was used for the present study. Administration of Cisplatin (5 mg/kg b.w.i.p) lead to significant ($P<0.001$) rise in the serum creatinine (from 0.89 to 3.62 mg/dl and from 0.99 to 3.56 mg/dl) and serum urea levels (from 29.29 to 55.96 mg/dl and from 32.73 to 59.06 mg/dl) indicating the induction of nephrotoxicity (Table1 and 2). Serum Creatinine levels reflect the rate at which the kidneys filter blood which is called as glomerular filteration rate. In case of malfunctioning of filteration of Blood through kidney, the glomerular filteration rate decreases, and the serum Creatinine level rises.

Table 1. Effect of administration of methanolic extracts of *P. kurroa* rhizome and Arogyawardhini bati on various biochemical parameters in Cisplatin induced renal damage.(Nephrocurative studies).

Group n=6/ group	Treatment regimen	% change in body weight	Blood urea (mg/dl) (% Reduction)	Serum creatinine (mg/dl)
1	Vehicle	7.31±1.05	29.29±1.58	0.89±0.09
2	Cisplatin 17th day (Toxicant)	- 11.71±1.32***	55.96±2.28***	3.62±0.46***
3	Cisplatin + Methanol extract 300 mg/kg b. w. p.o.	-6.67±2.03	45.98±1.28 (37.39)	2.45±0.24* (42.93)
4	Cisplatin + Methanol extract 600mg/kg b. w p.o.	5.90±1.44***	36.67±1.48** (72.35)	2.12±0.33** (55.09)
5	Cisplatin + Arogyawardhini Extract 600 mg/kg b.w.p.o	7.56±1.56***	32.37±3.12*** (88.48)	2.18±0.26** (52.74)
6	F_{cal}	35.51	8.37	10.75

Values are expressed as Mean ± S.E.M. (n=6) .The toxicant group is compared with vehicle group and the treated groups are compared with the toxicant group by One way ANOVA test followed by Bonferroni test with the toxicant group***P<0.001 **P< 0.01, *P<0.05.

F tab (4, 25) **P= 0.01 is 4.02; and *P=0.05 is 2.69

Table 2. Effect of administration of methanolic extracts of *P. kurroa* rhizome and Arogyawardhini bati on various biochemical parameters in Cisplatin induced renal damage. (Nephroprotective studies).

Group n=6/ group	Treatment regimen	% change in body weight	Blood urea (mg/dl) (% Reduction)	Serum Creatinine (mg/dl)
1	Vehicle	7.64±2.79	32.73 ± 2.02	0.99±0.20
2	Cisplatin 7th day (Toxicant)	-9.58±1.01***	59.06 ± 5.64***	3.56±0.33***
3	Cisplatin + Methanol extract 300 mg/kg b. w. p.o.	-3.43±0.45*	46.66 ± 4.59 (46.95)	3.07±0.19 (19.24)
4	Cisplatin + Methanol extract 600mg/kg b. w p.o.	6.11±0.72***	38.33± 2.47** (78.65)	1.82±0.42** (68.84)
5	Cisplatin + Arogyawardhini Extract 600 mg/kg b.w.p.o	5.92±0.45***	31.51 ± 3.15*** (104.60)	2.04±0.24** (60.27)
6	F_{cal}	29.39	8.93	10.95

Values are Mean ± S.E.M. (n=6) .The toxicant group is compared with vehicle group and the treated groups are compared with the toxicant group by One way ANOVA test followed by Bonferroni test with the toxicant group***P<0.001 **P< 0.01, *P<0.05.

F tab (4, 25) at P= 0.01 is 4.02; and at P=0.05 is 2.69

A marked reduction in the body weight by about 7.30 to 11.70 % was also observed due to administration of Cisplatin (5 mg/kg b.w.i.p). The histopathological studies of kidney obtained from the Cisplatin treated rats, revealed the nephrotic lesions, inflammatory sites and moderate granular degeneration of jaxtramedullary tubular epithelium (Fig.2.), when compared with the normal architecture of the kidney (Fig.1.). Thus, histopathological examination, reduction in the body weight and elevated levels of serum creatinine and serum urea confirmed the induction of nephrotoxicity in the animals treated with Cisplatin.

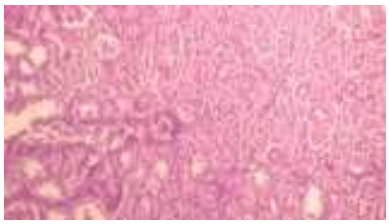

Fig-1.

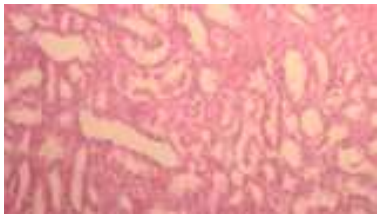

Fig-2.

Fig. 1. Photomicrograph of Healthy kidney showing normal glomeruli and tubules.

Fig. 2. Photomicrograph of kidney treated with Cisplatin, showing marked granular degeneration of jaxtramedullary tubular epithelium, tubular casts and inflammatory sites.

In case of curative regimen, after confirming the induction of nephrotoxicity, day 16 onwards, treatment with the ethanolic extract of *P. kurroa* and the formulation Arogyawardhini Bati was commenced.For nephroprotective studies, the animals were treated with the extract and the formulation for 6 days, followed by administration of Cisplatin, to evaluate the nephroprotective potential of the drugs under investigation. The parameters like, serum creatinine, serum urea, body weight and histopathological examinations were utilized for evaluation of nephrocurative and nephroprotective activity.

Administration of *P.kurroa* in the dose of 300mg/kg b.w.p.o revealed that there is no significant rise in the body weight, reduction in serum Creatinine and serum urea levels when evaluated for nephrocurative studies (Table.1.). Histopathological examination also indicated presence of marked granular degeneration of jaxtramedullary tubular epithelium, nephrotic lesions and inflammatory sites

with glomerular cast, as seen in cisplatin induced toxicity. Thus the results indicate that the dose of 300mg/kg/b.w.p.o could not recover the damaged kidneys

Nephrocurative effect of the extract against Cisplatin induced nephrotoxicity was further studied by increasing the dose of extract 600 mg/kg b.w.p.o. The results presented in Table.1 depict that there is significant (P<0.01) reduction of serum creatinine and serum urea by 55.09 % and 72.35 % respectively.

The loss of body weight due to Cisplatin toxicity, was also found to be significantly (P<0.001) regained by the treatment with the methanolic extract (600 mg/kg b.w.p.o) of the root. Histopathological examinations of the kidneys treated with the root indicated mild to moderate granular degeneration of jaxtramedullary tubular epithelium, mildly multifocal and a mild to interstitial nephratitis (Fig.3.).

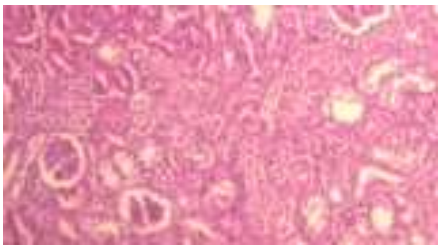

Fig. 3. Photomicrograph of kidney treated with *P. kurroa* extract (600mg/kgb.w.p.o,) and Cisplatin showing mild to moderate granular degeneration of jaxtramedullary tubular epithelium and interstitial nephratitis cells. (curative group).

From the results obtained by the treatment with the extract (600mg/kg b. w. p. o), it can be said that there is curative effect exhibited by the extract and indicates recovery of the kidney.

Treatment with the Arogyawardhini Bati (600 mg/kg b.w.p.o equivalent to *P.kurroa*) could also significantly (P<0.01) reduce serum creatinine from 3.62 to 2.18 mg/dl and serum urea levels from 55.96 to 32.37 mg/dl. The significant (P<0.001) regaining of the reduced body weight was also observed as compared to the toxicant group. Histopathological studies were also indicating the curative effect, as mild

to moderate granular degeneration of jaxtramedullary tubular epithelium; mildly multifocal and mild to interstitial nephratitis (Fig.4.) were observed in the sections of the kidneys.

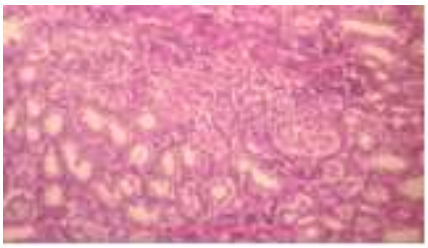

Fig 4. Photomicrograph of Kidney treated with Arogyawardhini bati extract (equivalent to 600 mg/kg b.w.p.o. of *P. kurroa* extract) and Cisplatin showing mild to moderate granular degeneration of glomeruli and tubular cells (Curative group).

In case of the studies involving evaluation of nephroprotective effect of the ethanolic extract at the dose of 300mg/kg b. w. p. o along with the toxicant viz. Cisplatin (5mg/kg b.w.i.p.), it was observed that there is no significant reduction in serum creatinine and serum urea levels also no restoration of body weight, (Table.2). The histopathological examination indicated presence of the granular degeneration of jaxtramedullary tubular epithelium and interstitial nephratitis. Hence, it can be inferred that the extract could not offer protection to the kidneys at the dose of 300mg/kg/b.w.p.o from Cisplatin.

Results of the studies on nephroprotective effects of the methanolic extract at the dose of 600 mg/kg b.w.p.o are presented (Table.2). It was observed that the extract could offer protection to the kidney against Cisplatin, as the there is significant ($P<0.01$) reduction in the serum creatinine from 3.55 to 1.82 mg/dl and serum urea from 59.058 to 38.33 mg/dl, as compared to the toxicant group.

Administration of the formulation, Arogyawardhini Bati, could also offer the protection against Cisplatin induced toxicity, which is revealed through the marked reduction in the values of the biochemical parameters viz, serum creatinine from 3.55 to 2.04mg/dl and serum urea from 59.06 to 31.51mg/ml, as compared to the Cisplatin group. Restoration of the body weight was also observed

(P<0.001) as compared to toxicant group .Mild to moderate granular degeneration of jaxtramedullary tubular epithelium, mildly multifocal, mild interstitial nephratitis was detected in the histopathological examination. (Fig.5-6).

Fig-5.

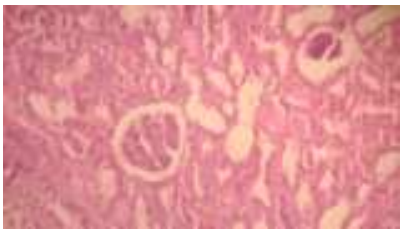

Fig-6.

Fig. 5. Photomicrograph of Kidney following treatment with *Picrorhiza kurroa* (600 mg/kg b.w.p.o.) in the Cisplatin- treated group showing normal glomeruli and tubular cells (Preventive group).

Fig. 6. Photomicrograph of Kidney following treatment with Arogyawardhini bati extract (equivalent to 600 mg/kg b.w.p.o. of *P. kurroa* extract) in the Cisplatin- treated group showing normal glomeruli and tubular cells (Preventive group).

In vitro evaluation of antioxidant property of *P. kurroa,* indicated that the drug has nitric oxide free radical scavenging effect [8]. Nitric oxide has been shown to play a vital role in Cisplatin-induced nephrotoxicity [9]. Hence, the probable mechanism of nephroprotection by *P. kurroa* and Arogyawardhini bati could be due to its antioxidant property and free radical scavenging property (fig 7-9). IC_{50} values of Ascorbic acid, ethanol extract and Arogyawardhini extract were found to be 54.07,206.69 and 117.23 µg/ml respectively .

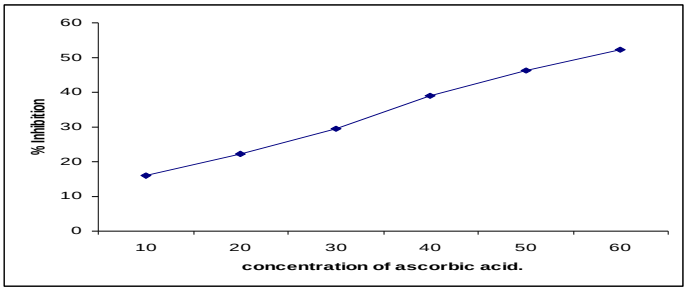

Fig . 7. Nitric oxide scavenging activity of Ascorbic acid.

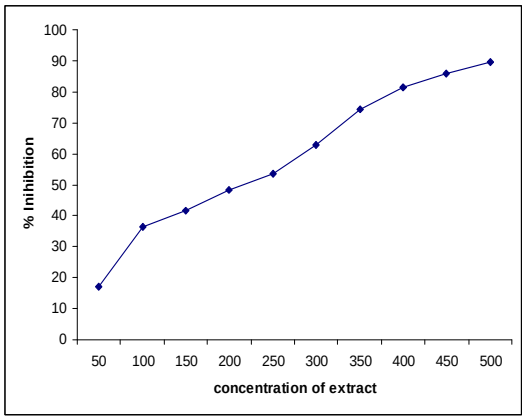

Fig. 8. Nitric oxide scavenging activity of *P. kurroa.*

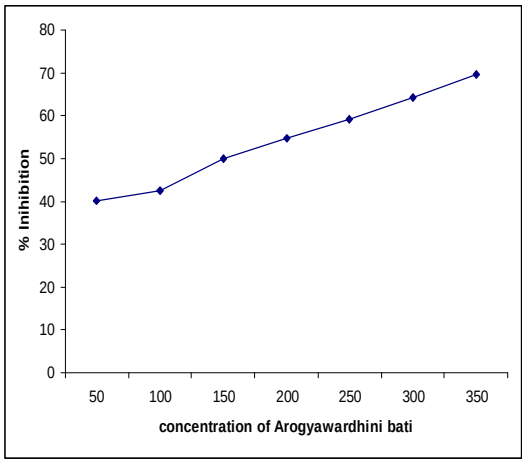

Fig . 9. Nitric oxide scavenging activity of Arogyawardhini bati

4. DISCUSSION

Cisplatin (Cis- diamino dichloro platinum II) is currently used as one of the most important therapeutic drugs in the treatment of wide range of solid tumors of head, neck, ovary and lung cancer. Cisplatin gets accumulated in the tubular epithelial cells of proximal kidney tubule, thus leading to nephrotoxicity. The nephrotoxicity is characterized by morphological destruction of intra cellular organelles and cellular necrosis [10].

Changes in the kidney architecture leads to impairment of the kidney function which is reflected in the elevated levels of the serum creatinine and blood urea levels. Cisplatin- induced renal impairment is evidenced by significant ($P<0.001$) increase in blood urea, serum creatinine, as well as acute tubular

necrosis that was evidenced through the histopathological examination of the kidney, due to administration of single dose of Cisplatin, in both the regimen of nephrocurative and nephroprotective effects. These changes persisted till the 16th day following the administration of a single dose of Cisplatin. Treatment with the ethanolic extract of *P. kurroa* and one of the Ayurvedic preparations viz. Arogyawardhini Bati could protect the kidney from the toxicity induced by Cisplatin, as there was no significant elevation observed in the serum creatinine and the blood urea levels. The nephroprotective effect was also confirmed by the histopathological examinations of the kidney, wherein the normal architect of the cellular structure is restored with absence of inflammatory casts. The ethanolic extracts of *P. kurroa* and Arogyawardhini Bati exhibited nephrocurative effect in the rats intoxicated with Cisplatin in the higher doses of 600 mg/kg b.w.p.o. as significant ($P<0.01$) reduction in the serum creatinine and blood urea levels are observed.

Histopathological examination of the kidneys of the rats treated with these drugs revealed absence of inflammatory casts and glomerular congestion, thus supporting the nephrocurative effect of the extract of *P. kurroa* and Arogyawardhini Bati. There is no significant difference in the values of the biochemical parameters and the histology of the kidney of rats treated with ethanolic extract of *P. kurroa* as compared to the control group indicate that the extract itself does not have any nephrotoxicity. A relationship between oxidative stress and nephrotoxicity has been well-demonstrated in many experimental animal models [11,18]. Thus, the studies indicate that the *P. kurroa*, a well known plant for offering protection to liver, can also be administered along with the drugs like Cisplatin, which is prescribed in the treatment of cancer to protect kidney as the treatment leads to the nephrotoxicity. The nephroprotective and the nephrocurative effects of an ayurvedic formulation viz. Arogyawardhini Bati is more pronounced as compared to the *P. kurroa* alone, which may be probably due to additive effects of the other drugs like Amala, which is rich in Gallic acid and Guggul which contains Guggulosterones.

5. CONCLUSION

The rhizomes of *P. kurroa* exhibited good nephrocurative and protective activity however, the formulation arogyavardhini bati could offer better protection probably due to the presence of tannin containing drugs. This is also supported by the better antioxidant activity exhibited by the formulation as compared to the rhizomes.

6. ACKNOWLEDGEMENT

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