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Nephroprotective Activity of Some Indigenous Medicinal Plants in Cisplatin Induced Nephrotoxicity

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Abstract

Aim: The aim of the present investigation is to study the nephroprotective activity of two indigenous medicinal plants. **Material and methods:** Dried leaves of *Alstonia scholaris* and *Centella asiatica* were procured from the medicinal garden and campus of Pharmacy College in the month of September, Bhopal, Madhya Pradesh, India. Around 500 gm dried leaves of *Alstonia scholaris* and *Centella asiatica* were coarsely powdered weighed and filled in Soxhlet apparatus for extraction. Wistar albino rats of either sex between 2 and 3 months of age weighing 150-200 gm were used which were procured from the central animal house. The acute oral toxicity studies were carried out according to the guidelines set by the Organization for Economic Co-operation and Development (OECD), revised draft guideline 423. Cisplatin (5mg/kg, *i.p*) was administered to the rats. The study was conducted for 15 days and the rats were divided into 12 groups (n=6). **Rsults:** Among all the extracts of *Centella asiatica*, methanolic extract showed a significant effect (P>0.01) as compared to other extracts. This shows that at these doses of 200 and 400 mg/kg the extract have preventive and protective potential against Cisplatin renal toxicity, suggesting it renal preventive role. Among all the extracts of *Alstonia scholaris*, dichloromethane extract showed a most highly significant effect (P>0.001) as compared to other extracts. Methanolic extract also showed significant effect (P>0.01) as compared to other extract. Petroleum ether showed almost negligible effect. **Conclusion:** The results obtained in this study have shown that extracts displayed significant nephroprotective activity in both acute and chronic conditions. Besides from the obvious therapeutic importance, these components would be useful in understanding the mechanism of diseases with higher levels of cellular and molecular level.

Keywords: *Alstonia scholaris*, dichloromethane extract, *Centella asiatica,* Gentamycin, Indigenous medicinal plants

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Introduction

Intense kidney harm or intense renal disappointment mirrors an expansive range of clinical presentations going from mellow damage to extreme harm that may bring about perpetual and loss of renal capacity. The occurrence of non-dialysis-requiring AKI is around 5000 cases per million individuals per each year and frequency of dialysis requiring AKI is 295 cases per million individuals per every year.[1] AKI has been demonstrated as independent risk factors for mortality and the Intensive Care National Audit Research Center (ICNARC) reported that AKI accounts for 10 % of all intensive care unit beds.[2] A meta-analysis of 13 cohort study revealed that AKI is one of the risk factor for chronic kidney diseases (CKD).[3] The patients who required dialysis and transplantation due to kidney failure are found to increase from year to year.[4]

Nephrotoxicity associated with drugs lengthens hospital stay, worsens prognosis, and limits the therapeutic benefit of the drugs. The proximal tubule is the major target of drug toxicity as it recovers 60% of the filtered load. A single molecule of toxins that is filtered and reabsorbed will pass through the proximal tubule more than 50 times a day and may lead to proximal tubular cell death.[5,6]

Plant extracts and phytochemicals are very beneficial in the treatment of various diseases and conditions.[7] Example of such plant is *Alstonia scholaris* and *Centella asiatica* are deciduous plant used medicinally for ulcers, cuts, sores, wounds, general body pains, hemorrhoids, diuretics, malaria and yellow fever.[8] Due to its diuretic,[1] anti-hyperglycemic[3] and anti – inflammatory properties,[9] the present study was aimed at exploring the nephroprotective effects of selected Indian medicinal plants in experimental rats.

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Material and Methods

Collection of Plants

Dried leaves of *Alstonia scholaris* and *Centella asiatica* were procured from the medicinal garden and campus of Pharmacy College in the month of September, Bhopal, Madhya Pradesh, India. Both crudes are washed properly through water and dried in shade for the further process.

Drugs and Chemicals

Gentamycin- 80mg/2ml was purchased from Ranbaxy, Cisplatin (Cytoplatin)- 50mg/ml was purchased from Cipla Ltd, Bovine serum albumin, DPPH (1,1 Dipheyl,2-Picryl hydrazyl), Sodium citrate, Methanol from SD Fine Chemicals Ltd; Hyderabad, India.

Kits - Urea Kit, Creatinine Kit from M S Excel Diagnostics Private Limited, Hyderabad.

Equipments

Digital Balance (SHIMA DZU, AX 200), UV/Visible Spectrophotometer (UV/win5 software), Tissue Homogenizer (REMI MOTOR), Laboratory cooling centrifuge (REMI R- 8C), Serum analyzer (Inkarp,ES-100), Trinocular Microscope (Labomed, IVU-3100),

B.O.D incubator (Barath biotech).

Extraction Method

Around 500 gm dried leaves of *Alstonia scholaris* and *Centella asiatica* were coarsely powdered weighed and filled in Soxhlet apparatus for extraction. First the powdered drug was defatted with petroleum ether (60°C-80°C); Defatted drug was then dried and again filled in soxhlet apparatus for successively extraction with dichloromethane, ethyl acetate, methanol and water as solvent. The extraction was carried out for a period of 72 hr. The extract obtained was dried in vacuum to remove excess solvent and were weighed for the determination of % yields.[11]

Preliminary Phytochemical Tests

Qualitative chemical tests of ethanolic and Methanolic extracts were subjected to various chemical tests to detect various phytoconstituents.[12]

Preliminary *In Vivo* Nephroprotective Zctivity

Selection of animals

Wistar albino rats of either sex between 2 and 3 months of age weighing 150-200 gm were used which were procured from the central animal house. All animals were housed in an animal room under normal condition of 25±1^{0}C, 12 hr light and dark cycle. The animals were allowed free to access commercial rat pallet diet (Lipton India Ltd, Mumbai, India) and water *ad libitum*. The bedding materials of the cages were changed every day. All the experimental procedures were carried out in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.[13]

Acute toxicity studies

The acute oral toxicity studies were carried out according to the guidelines set by the Organization for Economic Co-operation and Development[12], revised draft guideline 423.

Nephroprotective Activity of Different Extracts of *Centella asiatica* in Cisplatin Induced Nephrotoxicity

The treatment or grouping schedules were as follows.

Group-I: Normal Control

Group-II: Disease Control (Cisplatin induced toxicity, 5mg/kg, *i.p*)

Group-III: Pet ether extract treated rats in a dose of 200 mg/kg+ Cisplatin, 5mg/kg, *i.p*

Group-IV: Pet ether extract treated rats in a dose of 400 mg/kg+ Cisplatin, 5mg/kg, *i.p*

Group-V: DCM extract treated rats in a dose of 200 mg/kg+ Cisplatin, 5mg/kg, *i.p*

Group-VI: DCM extract treated rats in a dose of 400 mg/kg+ Cisplatin, 5mg/kg, *i.p*

Table 1: Extracts with their appearance & % yield (in gm) of *Centella asiatica*

S.No.	Extracts	Color of dried extracts	Consistency of dried extracts	% Yield (W/W)
1	Pet ether extracts of *Centella asiatica*	Dark Brown	Sticky	5%
2	Dichloromethane extracts of *Centella asiatica*	Dark Brown	Sticky	11%
3	Ethyl Acetate extracts of *Centella asiatica*	Dark Brown	Sticky	9%
4	Methanolic extracts of *Centella asiatica*	Dark Orange	Sticky	12%
5	Water extracts of *Centella asiatica*	Dark Brown	Sticky	8%

Table 2: Extracts with their appearance and % yield (in gm) of *Alstonia scholaris*

S. No.	Extracts	Color of dried extracts	Consistency of dried extracts	% Yield (W/W)
1	Pet ether extracts of *Alstonia scholaris*	Dark Green	Sticky	9%
2	Dichloromethane extracts of *Alstonia scholaris*	Dark Green	Sticky	13%
3	Ethyl Acetate extracts of *Alstonia scholaris*	Dark Orange	Sticky	7%
4	Methanolic extracts of *Alstonia scholaris*	Dark Brown	Sticky	8%
5	Water extracts of *Alstonia scholaris*	Dark Brown	Sticky	5%

Group-VII: EA extract treated rats in a dose of 200 mg/kg+ Cisplatin, 5mg/kg, *i.p*

Group-VIII: EA extract treated rats in a dose of 400 mg/kg+ Cisplatin, 5mg/kg, *i.p*

Group-IX: MeOH extract treated rats in a dose of 200 mg/kg+ Cisplatin, 5mg/kg, *i.p*

Group-X: MeOH extract treated rats in a dose of 400 mg/kg+ Cisplatin, 5mg/kg, *i.p*

Group-XI: Water extract treated rats in a dose of 200 mg/kg+ Cisplatin, 5mg/kg, *i.p*

Group-XII: Water extract treated rats in a dose of 400 mg/kg+ Cisplatin, 5mg/kg, *i.p*

Nephroprotective Activity of Different Extracts of *Alstonia Scholaris* in Cisplatin Induced Nephrotoxicity

Cisplatin is the highly effective chemotherapeutic agent widely used in the treatment of a variety of cancers. However, its clinical utility is limited due to its most common adverse effect i.e. nephrotoxicity in many patients.[14] Cisplatin (5mg/kg, *i.p*) was administered to the rats. The study was conducted for 15 days and the rats were divided into 12 groups (n=6).

On the 16th day blood was taken out from tail vein method from all groups assessed for renal function tests.[14]

The treatment or grouping schedules were as follows.

Group-I: Normal Control

Group-II: Disease Control (Cisplatin induced toxicity, 5mg/kg, *i.p*)

Group-III: Pet ether extract treated rats in a dose of 200 mg/kg+

Table 3: Qualitative chemical analysis of extracts by chemical tests

S. No	*Phytoconstituents*	*Chemical Tests*	*Alstonia scholaris*	*Centella asiatica*
1	Alkaloids	Wagner's test	+	+
		Dragendorff's test	+	+
		Mayer's test	+	+
		Hager's test	-	+
2	Amino Acid	Millon's test	+	-
		Ninhydrine test	-	-
3	Flavonoids	Shinoda test	+	+
		Alkaline reagent test	+	+
		Zinc hydrochloride test	+	-
4	Phenolics (Tannins)	Gelatin test	+	+
		Phenazone test	-	-
		Ferric chloride test	+	+
5	Protein	Biuret test	+	-
		Hydrolysis test	+	-
		Test with trichloroacetic acid	-	-
6	Triterpenoids & Steroids	Libermann -Burchard test	+	+
		Salkowski test	+	+
7	Carbohydrates	Benedict's test	+	+
		Fehling's test	+	+
		Molish's test	-	-
8	Anthraquinone glycosides	Borntrager's test	+	+
		Modified Borntrager'stest	+	+
		Baljet's test	-	-
		Legal's test	+	+
		Keller-killiani test	+	+

Cisplatin, 5mg/kg, *i.p*

Group-IV: Pet ether extract treated rats in a dose of 400 mg/kg+ Cisplatin, 5mg/kg, *i.p*

Group-V: DCM extract treated rats in a dose of 200 mg/kg+ Cisplatin, 5mg/kg, *i.p*

Group-VI: DCM extract treated rats in a dose of 400 mg/kg+ Cisplatin, 5mg/kg, *i.p*

Group-VII: EA extract treated rats in a dose of 200 mg/kg+ Cisplatin, 5mg/kg, *i.p*

Group-VIII: EA extract treated rats in a dose of 400 mg/kg+ Cisplatin, 5mg/kg, *i.p*

Group-IX: MeOH extract treated rats in a dose of 200 mg/kg+ Cisplatin, 5mg/kg, *i.p*

Group-X: MeOH extract treated rats in a dose of 400 mg/kg+ Cisplatin, 5mg/kg, *i.p*

Group-XI: Water extract treated rats in a dose of 200 mg/kg+ Cisplatin, 5mg/kg, *i.p*

Group-XII: Water extract treated rats in a dose of 400 mg/kg+ Cisplatin, 5mg/kg, *i.p*

Sampling and Biochemical Analyses for Estimation of Renal Parameters

For this method, the samples of the blood was withdrawn, collected in absence of sodium citrate and allowed to clot. The clotted blood was centrifuged for 10 minutes at 2500 rpm. The obtained clean and clear serum was refrigerated at 20^0C for the analysis of blood urea, serum creatinine (CR), and uric acid (UA) level by adopting & utilizing colorimetric assay kits as per given procedure.[14]

Estimation of Renal Parameters

Determination of creatinine

1. Equal volumes of reagent 1 and reagent 2 were mixed. Wait for 15 minutes before use.
2. Serum is preferred but heparinized plasma may be used. We used serum in this case.
3. The solution was mixed well and initial absorbance (A1) was read 20 seconds after mixing and final absorbance (A2) 80 seconds after mixing.
4. The results were calculated as follows:

$$\Delta A = A2 - A1$$

$$\text{Creatinine} = \frac{\Delta A \text{ of Test} \times \text{ Concentration of standard } (\text{mg/dl})}{\Delta A \text{ of Standard}}$$

Determination of urea

1. The reagent and Aqua-4 was allowed to get room temperature (15-300C).
2. The amount of Aqua-4 indicated on the label was added to dissolve the contents of each bottle of substrate and mixed gently. It was not shaking vigorously.
3. The absorbance change (ΔA) for the standard and unknown samples was determined by using the formula:

$$\Delta A = A2 - A1$$

Determination of Uric Acid

The reagents were mixed and kept at incubation for 5 minutes at 37°c the absorbance of standard and each test sample were read at 505 nm on biochromatic analyzers against reagent blank.

Statistical Analysis

The values are expressed in mean ± SEM. The results were analyzed by using one way analysis of variance (ANOVA) followed by Dunnet's "t" test to determine the statistical significance. $p< 0.05$ was chosen as the level of significance.

Results

Extractive Value Determination

Dried leaves of *Alstonia scholaris* and *Centella asiatica* were extracted using pet ether, dichloromethane, ethyl acetate, methanol and water [Tables 1 and 2].

Preliminary Phytochemical Screening

The preliminary phytochemical analysis revealed that different active constituent present in different extracts such as carbohydrates, proteins, amino acids, fat, oils, steroids, terpenoids, glycosides, alkaloids, tannins and other phenolics compounds [Table 3].

Acute Toxicity Studies of Plant Extracts

No toxic effects were observed at a higher dose of 2000 mg/kg body weight of Wistar rats. Hence, $1/10^{th}$ dose was selected as effective dose or therapeutic dose. The cut off value of 200 and 1/5 dose double of 400 mg/kg were selected for nephroprotective activity.

Table 4: Nephroprotective activity of different extracts in Cisplatin model

S.No.	Groups & Treatment	Biochemical Parameters		
		Blood Urea (mg/dl)	Serum creatinine in (mg/dl)	Uric acid (mg/dl)
1	Normal Control	53.20±2.34	0.684±0.01	1.634±0.26
2	Disease Control, 5 mg/kg	74.46±2.44	1.972±0.12	2.78±0.12
3	Pet ether extract, 200 mg/kg	70.45±2.43*	1.827±0.14*	2.61±0.16*
4	Pet ether extract, 400 mg/kg	67.31±3.32 *	1.733±0.18*	2.58±0.13*
5	DCM extract, 200 mg/kg	71.27±4.77*	1.902±0.19*	2.46±0.13*
6	DCM extract, 400 mg/kg	68.48±3.34*	1.812±0.12*	2.10±0.18*
7	EA extract, 200 mg/kg	72.59±3.28*	1.128±0.12*	2.48±0.16*
8	EA extract, 400 mg/kg	69.23±2.73*	1.833±0.14*	2.40±0.12*
9	MeOH extract, 200 mg/kg	65.42±3.46**	1.726±0.15**	2.38±0.17**
10	MeOH extract, 400 mg/kg	60.78±3.84**	1.865±0.14**	2.30±0.19**
11	Water extract, 200 mg/kg	70.25±3.64*	1.721±0.14*	2.59±0.13*
12	Water extract, 400 mg/kg	67.33±3.32 *	1.705±0.24*	2.47±0.14*

Values are representative of Mean±SEM (n=6). One way ANOVA with Dunnett's test. Where, *P<0.05 vs **P<0.01, Not significant. Toxic control *P*<0.05, *P*<0.01 vs control.

Table 5: Nephroprotective activity of different extracts in Cisplatin model

S. No.	Groups & Treatment	Biochemical Parameters		
		Blood Urea (mg/dl)	Serum creatinine (mg/dl)	Uric acid (mg/dl)
1	Normal Control	53.20±2.34	0.684±0.21	1.634±0.26
2	Disease Control, 5 mg/kg	74.46±2.44	1.972±0.42	2.78±0.52
3	Pet ether extract, 200 mg/kg	64.45±2.22*	1.522±0.35*	2.41±0.52
4	Pet ether extract, 400 mg/kg	61.37±2.82 *	1.501±0.24*	2.20±0.45*
5	DCM extract, 200 mg/kg	55.23±4.32**	0.932±0.24**	1.54±0.33**
6	DCM extract, 400 mg/kg	53.45±3.35***	0.692±0.15***	1.12±0.25***
7	EA extract, 200 mg/kg	65.54±2.24*	1.128±0.08*	2.12±0.16 *
8	EA extract, 400 mg/kg	63.26±3.43*	1.833±0.14*	1.44±0.07*
9	MeOH extract, 200 mg/kg	66.42±3.42**	1.828±0.35**	2.12±0.26**
10	MeOH extract, 400 mg/kg	61.78±2.82**	1.265±0.04**	1.77±0.05**
11	Water extract, 200 mg/kg	62.71±3.65**	1.128±0.62*	2.49±0.26*
12	Water extract, 400 mg/kg	60.25±2.32 **	1.865±0.04**	1.71±0.15**

Values are representative of Mean±SEM (n=6). One way ANOVA with Dunnett's test. Where, *P<0.05 vs **P<0.01, Not significant. Toxic control *P*<0.05, *P*<0.01 vs control.

Nephroprotective Activity of *Centella Asiatica* in Cisplatin Induced Nephrotoxicity

In the table, it is shown that there was variation and increase in the levels uric acid, creatinine and urea levels when compared to normal group animals. Pet ether, DCM, ethyl acetate, methanolic and water extracts were evaluated for the nephroprotective effects. This shows that at these doses of 200 and 400 mg/kg the extract have preventive and protective potential against Cisplatin renal toxicity, suggesting it renal preventive role. Among all the extracts of *Centella asiatica*, methanolic extract showed a significant effect (P>0.01) as compared to other extracts. Other extracts also showed nephroprotective effect however, it is very less as compared to all extracts [Table 4].

Nephroprotective Activity of *Alstonia Scholaris* in Cisplatin Induced Nephrotoxicity

In our protocol single injection (i,p) at a dose of 5mg/kg with Cisplatin resulted in the clear cut Renal toxic symptoms appeared causing acute renal failure. There was clear variation and alteration (increase) in the levels uric acid, creatinine and urea levels when compared to normal group animals. 200 and 400mg/kg dose of plant extract was able to bring down the elevated values of uric acid, creatinine and urea levels towards normal, when compared to toxic control groups. This shows that at these doses of 200 and 400 mg/kg the extract have preventive and protective potential against Cisplatin renal toxicity, suggesting it renal preventive role. Among all the extracts of *Alstonia scholaris*, dichloromethane extract showed a most highly significant effect (P>0.001) as compared to other extracts. Methanolic extract also showed significant effect (P>0.01) as compared to other extract. Petroleum ether showed almost negligible effect [Table 5].

Discussion

The kidneys are the significant focuses for the poisonous impacts of different chemical substances operators and thus drug-induced AKI is a frequent entity in clinical medicine. Drug induced renal toxicity is recognized as an important contributor to kidney diseases including AKI and CKD. The rate of nephrotoxic AKI is hard to gauge because of inconstancies of patient populaces and criteria of AKI. However, prospective cohort studies of AKI have documented the frequency of drug induced renal toxicity to be approximately 14-26 % in adult population. It is a significant concern in pediatric patients with 16% of hospitalized AKI being attributable primarily to a drug.[15]

The cisplatin chief's side effect is nephrotoxicity. An antioxidant set imbalance due to unnecessary ROS growth by cisplatin. This leads to GSH depletion and lipid peroxidation. So there are constant investigation running for compounds that gives nephroprotection in opposition to the renal impairment which caused via drugs such as cisplatin and gentamycin. In favor of this the allopathy offers no any remedial actions. Therefore it is very important that human being turns to the side of other substitute of medicine for solace. Thus in present study is an

effort to monitor the herbal extract for their nephroprotective activity.

Plant may serve as the alternative sources for the development of nephroprotective agent due to their biological activities. Several plants used in different system of traditional medicine have shown diuretic activity when tested on animal models. Some nephroprotective and diuretic drugs use in indigenous system are *Phyllanthus amarus*, *Mahonia aquifolium*, *Azadirachta indica* & many more medicinal plants etc.

In today's world, herbal medicinal plants have been highly valued throughout the globe as an important source of bioactive principles for the prevention and treatment of ailments.[16-18]

In the preliminary study, dried powders of all selected plants were extracted by using different solvents as per polarity charts. The extracts were dried and screened for the presence of various active constituents. The extracts showed the presence of alkaloids, terpenoids, flavonoids, glycosides, phenolic compounds, tannins, steroids and fatty acids. For the preliminary assessment, all the plant extracts were evaluated in different models of nephropathy in rats.

Acute toxicity study was carried out as per OECD guidelines for testing of chemicals. Paragraph 22 of the OECD guidelines 423 suggests two types of acute oral toxicity tests, i.e the limit test and the main test. The limit test is used in situations where the experimenter has information indicating that the test material is likely to be non-toxic. Acute toxicity study is by up and down method adopted by OECD (423, 2008a) to assess the safety of plant extracts selected for the present study. Animals were observed for 14 days (post administration) with special attention for first 4 hours, later all animals were carefully observed for signs and symptoms of toxicity continuously up to 24 hr and later up to 14 days. It was observed that all the animals were normal and active within two hours of post treatment. No other sign of toxicity or no mortality was observed and all the animals were survived for 14 days post administration of test drugs. Thus, the dose (2000mg/kg, *p.o*) was considered as safe.

Cis-diamminedichloroplatinum (II) (cisplatin) is an inorganic complex formed by an atom of platinum surrounded by chlorine and ammonia atoms in *cis* position of a horizontal plane. Since the accidental discovery over four decades ago, cisplatin has been widely used for chemotherapy. Although cisplatin has been a mainstay for cancer therapy, its use is mainly limited by two factors: acquired resistance to cisplatin and severe side effects in normal tissues, which include neurotoxicity, ototoxicity, nausea and vomiting, and nephrotoxicity. Cisplatin is a major anti neoplastic drug commonly used as front-line therapy for cancers such as small cell lung cancer, gut cancer, bladder cancer, stomach cancer, and ovarian cancer and germ cell tumors. Rats in which nephrotoxicity was induced by intra peritoneal administration of cisplatin (5mg/kg) showed characteristic signs of renal dysfunction and inflammation. The decline in renal function was reflected in the results, showing decrease in bodyweight, decreased urine output, increased levels of serum creatinine, BUN and decreased levels of serum total protein. Similarly decreased levels of urinary creatinine, creatinine clearance were observed.[19] This is an indication that different extracts of *Alstonia scholaris* and *Centella asiatica* in the present study have both preventive nephroprotection. Evidences state that in animal models cisplatin damages proximal tubules and cause mitochondrial swelling and nuclear pallor in the distal tubule. Thus the plant extracts have decreased oxidative stress injury.

Conclusion

The results obtained in this study have shown that extracts displayed significant nephroprotective activity in both acute and chronic conditions. Besides from the obvious therapeutic importance, these components would be useful in understanding the mechanism of diseases with higher levels of cellular and molecular level. These components could serve as lead molecules for development of prospective nephroprotective agents. Further detailed studies are required to elucidate the exact mechanism based on molecular and genetic level responsible for nephroprotective activity.

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