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Research Article

Nephroprotective Potential of Standardized Herbals Described In Ayurveda: A Comparative Study

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ABSTRACT

Acute kidney injury (AKI) is a severe threat to the modern drug treatment. There are only few drugs which are in clinical practice to revert the process of acute nephropathy. In Ayurveda, an ancient Indian system of medicine, several plants are designated for repair of kidney abnormalities but enough experimental data are not available. Here we have explored the nephroprotection of six such important medicinal plants. Water decoction of Crataeva nurvala, Pueraria tuberosa, Pterocarpus santalinus, Albizzia lebbek, Boerhaavia diffusa and Tribulus terrestris were standardized. AKI was induced by injecting cisplatin (6mg/kg BW, ip). Drug dose (50 mg/100 g BW, each) was given orally for five days to cisplatin induced AKI rats. On the 5th day urea, creatinine, creatinine clearance, lipid peroxidation and antioxidant status were measured in AKI rats. On 10th day following parameters in treated groups were determined and comparisons were drawn. Treated groups have reached the normal values of superoxide dismutase and catalase. Antioxidant properties were further correlated with reducing potential, tannins, flavonoids and phenolic content of the fraction; verified by Thin Layer Chromatography. Present investigation suggests that the water decoction of the selected herbals had shown significant protection against cisplatin induced AKI.

Keywords: Antioxidants, AKI, Cisplatin, Medicinal plants.

INTRODUCTION

Cisplatin is one of the widely used drugs for the treatment of solid tumors in the various forms of cancer. Nephrotoxicity of cisplatin (cis-diamminedichloroplatinum II) limits its clinical use [1]. Effects on renal tissue are healed naturally by hydration therapy but very often it ends up in the acute kidney injury. Renal failure is the main cause of death in cisplatin (CP) induced nephrotoxicity [2]. Several molecular mechanisms have been found to be involved in nephrotoxicity such as oxidative stress, inflammation and apoptosis. Cisplatin-induced acute renal tubular epithelial cell damage is one of the most important effects [3]. It acts by three mechanisms; oxidative stress, inflammatory reactions in the cells of glomerulus and tubular apoptosis in the kidney tissue since it is highly nephrotoxic in dose dependent manner [4]. Cisplatin is a coordinate complex of platinum. Platinum begins to accumulate in the epithelial tubular cells leading to the oxidative stress which in turn causes increase in the levels of IL-6, TNF-α [5]. It causes steep rise in molecular marker levels of IL-β, iNOS, ICAM-1, p53, NF-kB etc [6, 7]. Caspase-3 directly acts on BCl_2 causing its release from the mitochondria leading to apoptosis and finally death of the cells. There is an intensive study in the medical world to develop strategies to reduce its nephrotoxic effects. Various plants have been used in the Ayurvedic system to ameliorate the toxic effect of different nephrotoxic complications [8]. Here we have demonstrated the comparative study of the effects of six plants widely used in Indian Ayurveda. Nephrotoxicity of CP was evidenced by decrease in the Body weight, increase in kidney/body ratio and decrease in the percent survival of rats. Toxicity effects were confirmed biochemically by measuring the kidney function parameters in serum and level of antioxidant enzymes in blood hemolysates and kidney homogenates. Brief description of the nephroprotective plants are given below:-

Varun (*Crataeva nurvala*, Family-Capparidaceae)

Bark of the Varun is cytotoxic and contraceptive; used in kidney bladder stones, urinary disorders, fever, vomiting and gastric irritation. Roots and bark have laxative and lithontripic property; increases appetite and biliary secretion [9]. Varun bark contains various constituents like saponins, flavonoids, sterols, glucosilinates, ceryl alcohol, friedelin, cadabicine diacetate, lupeol, betulinic acid and diosgenin [10, 11].

Bidarikand (*Pueraria tuberosa*, Family-Fabaceae)

Tuber of this plant is widely used in different Ayurvedic formulations [12, 13]. They are generally used as tonic and laxatives. Tuber contains flavonoids such as daidzin, puerarin, genistin, puerarone, tuberosin, biochanin A, biochanin B [14, 15, 16] etc. It also contains coumstan (tuberostan), puerarostan, epoxychalcanol (puetuberosanol) and pterocarpanoids which are very specific to this plant [17].The plant tuber is nephroprotective as shown our earlier reports [18].

Lalchandan (*Pterocarpus santalinus* Linn., Family-Fabaceae)

Heartwood extract of Lalchandan is used as an anti-inflammatory, wound healing, analgesic and for diseases related to the blood. Its external applications are recommended for skin diseases and ulcers [19]. Its heartwood is enriched with tannins is an effective medicine which is rich in santalin, pterocarpin, lignins named as sabanin, carosednin, dibenzylghitylo-O-lactone, isoflavone glycosides namely 4,5 dihydroxy 7-op methyl isoflavone, 3-OH β- glucoside, auron glycosides, pentacyclic triterpenes namely 3-keto otenane. It is also a rich source of flavonoids, isoflavonoids, phenanthrenedione and chalcones [20]. Its water decoction is used as a medicine suggesting the efficacy of its polar phytochemicals [21]. Some of the earlier reports have shown its inhibitory role in LPS induced TNF-α expression on macrophages [22]. Presently there are no reports are available regarding its nephroprotective potential.

Shirish (*Albizzia lebbek*, Family-Fabaceae)

Shirish bark is well known for its anti-allergic and anti-asthmatic properties [23]. Its stem contains five sugars; D-glucose, D-galactose, D-arabinose, D-xylose and D-mannose. It also contains six amino acids viz glycine, alanine, valine, phenylalanine and leucine [24]. It is an important source of chemicals like melacacidin, sterols, flavonoids, saponins etc. It acts as a relieving agent in rectal inflammation, treating ulcers associated with piles, controlling bleeding and enhancing healing processes in inflamed mucous membranes [23]. It is proved to be an effective anticancerous agent in recent studies [25].

Punarnava (*Boerhaavia diffusa*, Family-Nyctaginaceae)

Roots of this creeper are widely used to treat internal inflammation of all kinds [26]. It contains various constituents like alkaloids (punarnavine), rotenoids (boeravinones A-F) and other phytochemicals. The most interesting metabolites from therapeutic point of view are the rotenoids known as boeravinones (A-F) and punarnavoside, a phenolic glycoside present in the roots [27]. Boeravinones (A-F) are very specific to this plant.

Chhota Gokshuru (*Tribulus terrestris*, Family-Zygophyllaceae)

Chhota Gokshuru is used as a tonic and an aphrodisiac. It has diuretic and uricosuric effects [28, 29]. It has been widely used in folk medicine throughout the history for conditions such as impotency, edema, rheumatism, hypertension, and kidney stones [30, 31]. Since kidney diseases are considered as pitta abnormality in Ayurvedic system addressing [8]. So we explored the potential effect of this plant on acute kidney injury experimental model. Late Prof. S N Tripathi of Department of Kayachikitsa, Institute of Medical Sciences, Banaras Hindu University had used this plant in management of nephritic syndrome in Polyherbal formulation developed by him in 1984, which is still in chemical use (personal communication).

Here we have tried to explore the nephroprotective potential of the medicinal plants addressed in Ayurveda. Mechanisms underlying the protective effects were investigated through intense study of the oxidative stress and antioxidants. Our study gives very crisp idea about the comparative effect of the selected important herbal plants.

EXPERIMENTAL SECTION

Materials

Chemicals and reagents were obtained from Sigma Aldrich chemical Co. (St. Louis, USA). Cisplatin was obtained from Fresenius Kabi Oncology Ltd. HP, India. Dried plant materials were collected from the Ayurvedic pharmacy, Banaras Hindu University, Varanasi, India. Authentication of the herbs were done by Prof. K. N. Dwivedi of the Department of Dravyaguna, IMS, BHU by letter no Da/13-14/139 comparing the characteristics of the plant mentioned in botanical texts and other floras [31]. All experimental animals were maintained in proper hygienic

condition. Animal experimentation protocol were approved by Animal Ethical Committee, IMS, BHU by letter no Dean/2012-13/192.

Preparation of water decoction of the herbals
Fresh herbal plant parts were collected from Ayurvedic garden, BHU and washed thoroughly. Fresh and infection free parts were isolated and shade dried. Twenty grams of mechanically made coarse powder of each plant were taken and it's four times water was added. Mixture was boiled till the one fourth of volume remained in the container. Water decoction was sieved with muslin cloth to remove the unwanted particles. Oil and various components were found to be present in the decoction while volatile components were absent.

Standardization of the water decoction fractions:

Preparation of Ethylaceate and Butanolic fraction for TLC fingerprinting
Aqueous fraction of each plant was mixed properly with equal amount of pure ethylacetate and vortexed vigorously for 15 minutes. After centrifugation at 1500 rpm, upper layer of ethylacetate was taken in a separate tube. Then, ethylacetate fraction containing various constituents were concentrated on waterbath making one-tenth of the initial volume. Further the bottom layer (that is remaining aquoeus layer) was again mixed and vortexed properly with same amount of pure butanol for 15 minutes. After centrifugation at 1500 rpm, upper layer of the butanol was separated out. TLC fingerprinting analysis was done in both the fractions for the presence of phytoconstituents.

Determination of Reducing potential of the plants
Reducing potential was determined according to the described method [32, 33]. High absorbance of the reaction mixture indicates high reducing power. Butylated hydroxy toluene (BHT) was used as a standard for comparison. Reducing power was measured by varying the concentration of the extracts and the contact time. It shows the overall antioxidant activity of the plant.

Experimental design, animal groupings and the drug treatment
Healthy animals were randomly divided into eight groups (n = 6).
•Group 1- Normal control rats administered orally with equivalent volume of normal saline.
•Group 2 – Rats were administered with single dose of cisplatin (6 mg/kg, ip).
•Group 3A - CP treated rats given with aqueous fraction of *Crataeva nurvala* bark (50 mg/100g BW).
•Group 3B - CP treated rats given with aqueous fraction of *Pueraria tuberosa* tuber (50 mg/100g BW).
•Group 3C - CP treated rats given with aqueous fraction of *Pterocarpus santalinus* heartwood (50 mg/100g BW).
•Group 3D - CP treated rats given with aqueous fraction of *Albizzia lebbek* (50 mg/100g BW).
•Group 3E - CP treated rats given with aqueous fraction of *Boerhaavia diffusa* (50 mg/100g BW).
•Group 3F - CP treated rats given with aqueous fraction of *Tribulus terrestris* (50 mg/100g BW).

Sixty four healthy male and female albino rats (100-200 g weight) of Charles Foster strain were chosen and divided into groups (n = 8). Vehicle, cisplatin and aqueous fractions were given according to the above experimental design. Cisplatin induced nephrotoxicity is a standard experimental model for AKI. CP was given to 3 hrs fasted rats (6mg/kg BW, ip) before and after which induced acute kidney injury following acute renal failure showing the rise in blood urea and creatinine on 5th day. Few rats were died initially due to extreme toxicity of the CP; while few others were died in the drug treated group also showing that in case of extreme toxicity, herbal treatment is not so effective. Group one served as normal control and received normal saline throughout the experiment. Group two (untreated) rats received single dose of CP interapritoneally. Other groups were received single dose of CP which resulted in significant rise in serum creatinine, urea, renal malondialdehyde and decline in the creatinine clearance. Toxicity of CP was evidenced by the decrease in body weight, increase in kidney/body weight ratio and decrease in cumulative percent survival of rats. Toxicity was confirmed biochemically by measuring kidney function parameters and oxidative stress markers. One week before the CP administration; rats were caged individually in a metabolic cage to obtain 6 hrs urine collection for normal values of creatinine clearance.

Herbal treatments were administered orally for 5 days at a dose of 50 mg/100g BW daily of each fraction from the 6th day of CP treated rats. After treatment, urinary functional parameters [34] were analysed; blood as well as kidney tissues were isolated and analyzed after sacrificing the rats. Parameters such as serum urea [35], serum creatinine (Cr) [36], plasma lipid peroxidation (LPO) [37], catalase (CAT) [38] and superoxide dismutase (SOD) [39] activities were determined. Drug treated rats showed lower blood urea and creatinine as compared to the untreated rats. In addition, treatment with CP resulted in development of a functional change in the kidney while no marked tubular necrosis was observed. Tubular dilation was seen in the kidney of rats with cloudy swelling. There were no such marked changes in the structural parameters. Rats were sacrificed on 10th day of cisplatin administration. Kidney was preserved at -80°C for antioxidant analysis through RT-PCR. 10% homogenate was prepared in

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phosphate buffer saline and above antioxidant parameters were determined at optimum temperature to ensure accuracy during the experiment.

Protein estimation
Protein content was estimated by Bradford assay (Sigma, USA) using bovine serum albumin (BSA) as standard.

Reverse transcriptase PCR (RT-PCR)
RNA extraction from kidney tissue was done by using Trizol reagent (Himedia Mumbai, India). Five microgram of total RNA was reverse transcribed with superscript II RNase H-reverse transcriptase (RT) using random hexamers according to the manufacturer's instructions (fermentas). The primer for rat SOD: forward (F) 5'- TCT AAG AAA CAT GGC GGT CC-3'; reverse(R)5'-CAG TTA GCA GGC CAG CAG AT-3'and for rat GAPDH forward (F):5'-AGT GAG GAG CAG GTT GAG GA-3'; reverse(R) 5'- CAG TTA GCA GGC CAG CAG AT-3'. The PCR product length of rat SOD and GAPDH were 387 BP and 244 BP respectively. Amplification was carried out in 2ul c-DNA, 2mmol/L dNTPs, 1.5 mmol/L MgCl2, 0.5 µmol/L of primer, 0.1 µmol/L of each primer (for GAPDH) 1U Taq DNA polymerase (Genaxy scientific PVT.LTD). The steps for SOD: initial denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 45 sec, 55°C for 30 sec, 72°C for 1.3 min and final extension at 72°C for 10 min. The steps for GAPDH : initial denaturation at 94°C for 3 min, followed by 35 cycles of 94°C for 30 sec, 58°C for 30 sec , 72°C for 45 sec and final extension at 75°C for 5 min. Amplified products were separated with 2% agarose gel electrophoresis. The bands were identified based on the product size using 100-bp ladder. The optical density of SOD were determined with alpha imager (Bio-Rad), and expressed as the ratio against GAPDH. All RT-PCR experiments were performed in triplicate from at least three independent treatments.

RESULTS AND DISCUSSION

Graphical representation of Kaplan-Meier survival curve for water decoction treated of different plants
This graph gives a brief idea about the probability of the surviving rats in the experiment. Rats were given different doses for ten days consecutively and provided full access to diet and water. Selected plants doses with survival probability and the number of rats surviving after giving the cisplatin dose was depicted in the graph (Fig. 1). Few rats started to die after 5th day day cisplatin treatment as evidenced in the curve.
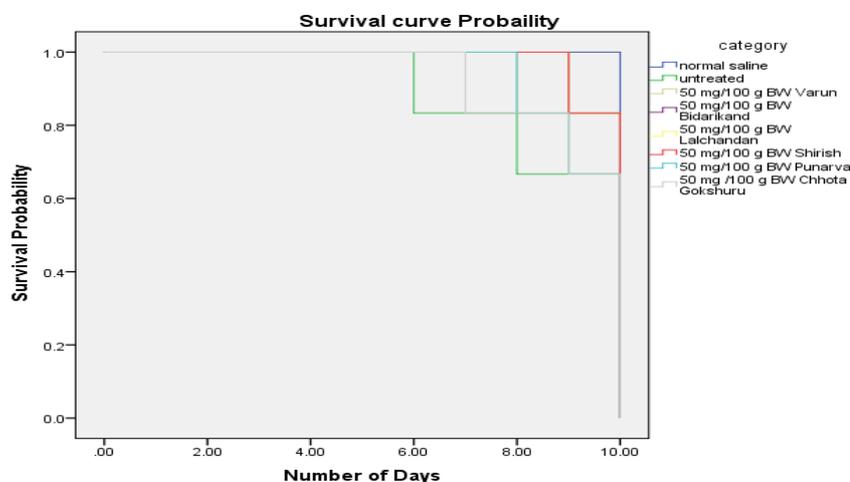

Figure 1: Kaplan-Meier Survival graph for untreated and treated groups in CP induced AKI rats

Fig: 1 Survival chart of rats for normal group, untreated group and treated group ie Varun, Bidarikand, Lalchandan, Shirish, Punarnava and Chhota Gokshuru with 50 mg/100 g BW each. They are denoted by different colours of the lines. Some treated group rats were died on the 7th day, 8th and 9th day showing that excessive damage to kidneys cannot be simply reversed by administration of herbals.

TLC Fingerprinting and analysis
TLC profile shows the antioxidant, reducing potential correlation. Most of the values of the lambda max are similar with one another depicting uniformity in chemical characteristics (Table No 1) and their similar functional metabolism in toxicity study. Phytoconstituents (eg alkaloids & tannins, flavonoids) are present in the plant as it is indicated by R_f values in the TLC profile and UV spectrum fingerprint. Alkaloids show Rf value at 0.52 and tannins at 0.69 as compared with the standard drug.

Table No 1: TLC analysis and λmax of water decoction of the selected plants

S.No	Medicinal plants	R$_f$ values[*]				λ$_{max}$[*] (long UV range)
		Ethylaceate fraction {solvent system, ethylaceate: methanol (8.5:1.5)}		Butanolic fraction {solvent system, benzene: methanol (8.5:1.5)}		
		Detection by iodine chamber	Detection by UV lamp	Detection by iodine chamber	Detection by UV lamp	
1	Varun (*CN*)	0.21, 0.71, 0.73, 0.95	0.21, 0.71, 0.73, 0.95	0.29, 0.33, 0.58, 0.85	0.32, 0.38, 0.61, 0.89	228 nm
2	Bidarikand (*PT*)	0.21, 0.71, 0.57, 0.96	0.24, 0.46, 0.74 0.98	0.61, 0.64, 0.99	0.11, 0.68, 0.98	227 nm
3	Lalchandan (*PS*)	0.35, 0.78, 0.90, 0.99	0.38, 0.64, 0.95, 0.99	0.30, 0.58, 0.65, 0.99	0.32, 0.61, 0.80, 0.99	218 nm
4	Shirish (*AL*)	0.21, 0.69, 0.70, 0.96	0.23, 0.46, 0.71, 0.97	0.59, 0.67, 0.98	0.59, 0.75, 0.90	230 nm
5	Punarnava (*BD*)	0.17, 0.57, 0.61, 0.96	0.13, 0.52, 0.64, 0.98	0.29, 0.44, 0.98	0.47, 0.89, 0.99	221 nm
6	Gokshuru (*TT*)	0.69, 0.73, 0.96, 0.86	0.34, 0.69, 0.78, 0.87, 0.96	0.30, 0.54, 0.60, 0.99	0.32, 0.56, 0.61, 0.93, 0.95	232 nm

Data[*] Were Expressed as the Mean of Triplicate.

Reducing potential of the herbals

Plants with good reducing potential are also good at free radical scavenging activity showing positive correlation with the antioxidant activity of the plants (Table No 2). The increasing reducing potential with increasing dose in concentration as compared to the standard indicates good reducing potential.

Table No 2: Reducing potential[a] of water decoction of the selected plants.

Conc. µg/ml	CN	PT	PS	AL	BD	TT	BHT (std compound)
100	0.034±0.010	0.022±0.009	0.105±0.012	0.19±0.011	0.088±o.011	0.032±0.010	0.251±0.054
300	0.101±0.022	0.045±0.012	0.145±0.018	0.289±0.022	0.101±0.015	0.09±0.019	0.348±0.064
500	0.167±0.011	0.111±0.022	0.239±0.012	0.371±0.016	0.167±0.011	0.15±0.022	0.631±0.053
1000	0.235±0.015	0.201±0.032	0.336±0.019	0.526±0.015	0.258±0.022	0.233±0.034	0.858±0.015
3000	0.305±0.067	0.303±0.039	0.426±0.023	0.676±0.016	0.358±0.034	0.333±0.024	1.138±0.025
5000	0.402±0.055	0.411±0.015	0.586±0.043	0.769±0.017	0.459±0.059	0.445±0.019	1.118±0.019

Data[*] were Expressed as the Mean of Triplicate.

Changes in urea and creatinine levels

Most important limiting factor in cisplatin use is its side effect on the physiology of kidney. Nephrotoxicity in experimental model was characterized by the elevation of serum markers (urea and creatinine), deterioration of renal functional parameters are shown in the Table No 3.

Table No 3: Comparison of urea and creatinine among groups (mean±SD) of cisplatin induced AKI rats

S.No	Group	Experiment	Urea (mg/dl)	Creatinine (mg/dl)
1	1	*Normal*	32.58±3.56	0.13±0.06
2	2	Exp cont	82.84±12.55*	0.84±0.11
3	3A	Varun (*Createva nurvula*)	66.66±10.72	0.60±0.05
4	3B	Bidarikand (*Pueraria tuberosa*)	50.29±11.98	0.44±0.08**
5	3C	Lalchandan (*Pterocarpus santalinus*)	36.86±8.96**	0.61±0.03
6	3D	Shirish (*Albizzia lebbek*)	56.16±9.26**	0.69±0.02
7	3E	Punarnava (*Boerhaavia diffusa*)	93.57±10.89**	1.38±0.06
8	3F	Chhota Gokshuru (*Tribulus terrestris*)	71.47±11.78**	0.81±0.07

Values are significantly different (*compared to control and **compared to cisplatin group; P < 0.01) within the same column.

Changes in the body weight

Cisplatin administration caused rapid decline in the body weight (Table No 4). There was sharp decrease in the body weight with hypertrophy of the kidney as shown in the table by kidney index values. Reduction in body weight by CP treatment is due to the injured renal tubules following the loss of tubular cells to reabsorb water leading to dehydration and decrease in body weight. Increased urinary volume might also be the reason behind the loss of body weight.

Table No 4: Comparison of body weight and kidney index among groups (mean ± SD) of cisplatin induced AKI rats

Groups	Experiments	Basal weight, 0th day	Initial weight, 5th day	Final weight, 10th day	Kidney index
1	Normal control	120±3.45	123±2.56	128±2.65	3.15±0.70
2	Untreated (Exp control)	112±1.65[a]	95±4.45[a]	83±3.42[a]	5.16±0.15[a]
3A	Varun	118±1.25[b]	90±3.78	89±3.21[b]	3.65±0.34[b]
3B	Bidarikand	94±2.31[b]	69±2.64	71±1.98[b]	4.14±0.45[b]
3C	Lalchandan	92±2.15[b]	74±2.12	76±2.74[b]	3.55±0.23[b]
3D	Shirish	100±4.51[b]	79±2.32	79±3.23[b]	3.98±0.21[b]
3E	Punarnava	119±4.32[b]	95±2.89	91±3.21	4.44±0.16[b]
3F	Chhota Gokshuru	114±3.82[b]	94±3.27[b]	86±3.28[b]	4.63±0.29[b]

Values are significantly different ([a]compared to control, and [b]compared to cisplatin group; p < 0.01) within the same column.

Antioxidant levels in blood hemolysates of drug treated animals

Decline in antioxidants aggravates the levels of reactive oxygen species and prevented the kidney protection against CP toxicity. In drug treated rats for five days, there was significant prevention in the rise of plasma LPO. In normal control LPO was 0.013 while in experimental control it increases to 1.023 nMol/mg of protein. Maximum inhibition was found in lalchandan followed by varun. Although there was significant inhibition in LPO in all the plants ranging 45% to 65% but Lalachandan showed best results (78.6%) followed by Bidarikand 76.6%. In experimental control rats blood SOD and CAT were significantly decreased but in extract treated rats this reduction was reversed to normal. Percentage of reversal was maximum in *Pterocarpus santalinus* Linn. followed by *Pueraria tuberosa* and *Albizzia lebbek* (Table No 5). In Punarnava and Chhota Gokshuru, reversal was not very high. It is important to note that maximum inhibition in LPO was found in Varun but SOD and CAT were not very high. It is higher in case of Lalchandan, Shirish, Bidarikand and Varun.

This could be linked to high direct free radical scavenging of the extract since flavonoid content was very high; this supports the decrease in LPO. *Pterocarpus santalinus* (PS) showed highest reversal of raised serum urea followed by the sequence: *Crataeva nurvala, Albizzia lebbek, Pueraria tuberosa, Boerhaavia diffusa and Tribulus terrestris*. However creatinine reversal response was observed in sequence: *Pterocarpus santalinus* Linn, *Albizzia lebbek, Crataeva nurvala, Pueraria tuberosa, Boerhaavia diffusa and Tribulus terrestris*. LPO increases significantly in experimental control but it decreases gradually in drug treated rats showing the healing property induced by antioxidants.

Table No 5: Effect of different plant extracts on antioxidant enzymes in blood hemolysates of cisplatin induced AKI rats

Groups	Experiment	% change in drug treated group with respect to the 5th day of CP treatment		
		Blood SOD	Blood CAT	Plasma LPO
1	Normal control	0.56±0.06	1.45±0.04*	0.013±0.002
2	Untreated (Exp control)	-131.78±5.15**	-79.49±2.78	1.023±0.07
3A	Varun	+12.54±0.98*	+6.12±0.95	0.387±0.04
3B	Bidarikand	+63.16±3.67*	+84.69±4.45*	0.257±0.05
3C	Lalchandan	+115.79±6.15	+163.67±6.12*	0.237±0.06
3D	Shirish	+65.52±3.12*	+80.32±2.19	0.392±0.02*
3E	Punarnava	+16.84±0.98*	+27.86±1.21*	0.448±0.04
3F	Chhota Gokshuru	+4.21±0.19	+9.39±0.93	0.547±0.03*

*P value is significant at the 0.01 level (2-tailed) **. P value is significant at the 0.05 level (2-tailed)*
*. Values on 5th day of CP (mean SD (n=6): SOD =0.12 U/mg of Hb, Catalase=9.18 U/mg of Hb,
LPO=1.03nmol/mg of protein; -ve value shows decrease and +ve value shows rise in the activity.

Antioxidant levels in kidney homogenates of the drug treated animals

Antioxidants are the defence mechanism of the body to be affected. Enzymes catalase and superoxide dismutase alteration occurs initially, there sharp decline in their activity. It is reported that CP induced depletion of these enzymes are due to the loss of copper and zinc, which are essential for SOD activity [40]. As a result cellular mechanism gets disturbed and imbalance lead to the membrane peroxidation. Here in untreated animals decrease in antioxidant level occurred. While in drug treated animals the level of these enzymes increases significantly, herbals Varun, Bidarikand, Lalchandan showed better response in ameliorating the effect of cisplatin induced AKI (Figure 3).
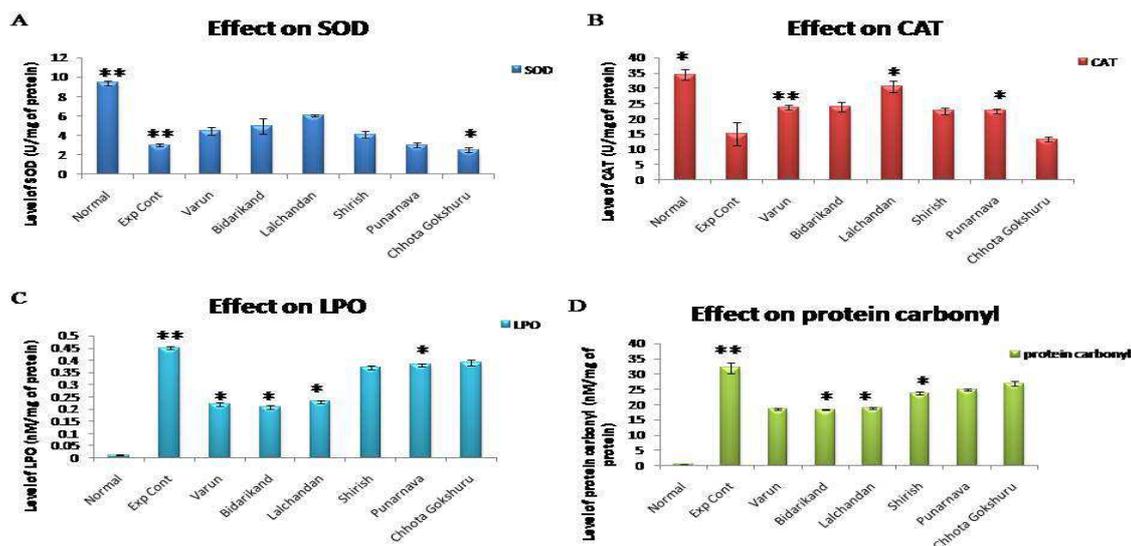
Comparision of different plants water decocotion on cisplatin induced AKI rats. A) It shows effect on SOD in comparision to normal and experimental control. B) Effect on catalase in normal and drug treated. C) Effect on lipid perioxidation in normal, exp cont and drug treated. D) Effect on protein carbonyl normal, expe cont and drug treated.

Urine analysis

Urinanalysis was done to monitor the kidney function by using test strip provided by Aggape kit and its relative semiautoanalyser. Since cisplatin caused polyuria initially as a body adaptation to remove excess platinum complexes deposited in the glomerulus (Table No 6).Treatment with the herbals caused significant change in the creatinine clearance as indicated in Fig 2. Herbal treatment brought the pH from alkalinity to normal level (5.5 to 7.0).

Table No 6: Effect of extract treatment on urinanalysis in cisplatin induced AKI rats

Groups	Experiment	Urine output (ml/24 hr)	Urine protein	Urinary MDA (nmol/mg Cr)	pH
1	Normal control	7.89 ± 0.40	-ve	0.06±0.01	6.5
2	Exp control	13.45± 0.20*	3+,>300mg/dl	2.06±0.09	9.0*
3A	Varun	8.78 ± 0.30†	2+,>100mg/dl	1.03±0.08	8.0
3B	Bidarikand	8.89 ± 0.35†	1+,>30mg/dl	1.12±0.07	7.5
3C	Lalchandan	8.19 ± 0.40†	-ve	0.91±0.06	6.5
3D	Shirish	9.25 ± 0.50†	2+,>100mg/dl	1.05±0.07	7.5
3E	Punarnava	10.62 ± 0.50†	2+,>100mg/dl	1.02±0.08	8.0
3F	Chhota Gokshuru	11.51 ± 0.30†	2+,>100mg/dl	1.05±0.07	8.0

*Values are Significantly Different (*Compared to Control, and † Compared to Cisplatin Group; p < 0.01) within the same column.*
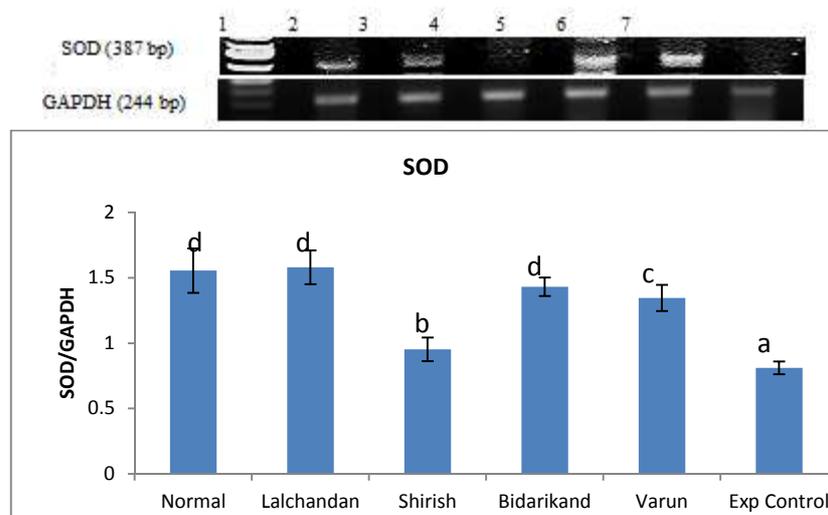
RT-PCR

Fig. 1: mRNA expression of SOD in rat kidney ;

Lane 1-DNA (100 bp) Ladder, Lane 2-Normal Control), Lane 3-Lalchandan treated, Lane 4-Shirish treated, Lane 5-Bidarikand treated, Lane 6-Varun treated, Lane 7-Experimental Control. The values are the mean ± SD. Different superscripts designate significant differences (p < 0.05).

mRNA expression of the enzyme SOD verifies the results as observed in case of kidney homogenates, Lalchandan and Bidarikand showed better response in the drug treated cisplatin rats in comparision to experimental control rats. In contrast to biochemistry of SOD, the mRNA expression of Varun was also found to be significantly higher than experimental control. Thus, some transcriptional changes may be involved here, needs more study at molecular level.   Nephroprotection and antioxidant properties of these plants are closely associated with different class of compounds found in the water decoction. Other herbals also causes rise in the antioxidants level. Medicinal plants with antioxidant properties like *Ginkgo biloba* [41, 42], *Rubia cordifolia* [43], *Echina pallidum* [44], *Pongamia pinnata* [45] and *Tinospora cordifolia* [46] showed significant protection against cisplatin induced nephrotoxicity. Therefore, herbal formulations can be designed at optimum concentrations could produce several beneficial effects.

Statistics
Statistical analysis was performed by using by One Way ANOVA and t-test using IBM SPSS (version 20.0) and Microsoft excel 2010. Descriptive statistics for the numerical data were calculated as mean and standard deviation. P-values less than 0.05 were considered statistically significant in the considered experiment.

CONCLUSION

Cisplatin induced nephrotoxicity was reversed significantly by the aquoeus fraction of *Pterocarpus santalinus*, probably via its antioxidant activity. Its aqueous fraction conferred maximum protection which suggests that semipolar antioxidant principles might be responsible for the observed effect. Still in the treatment modalities of nephrotoxicity, hydration therapy is considered as the most important treatment in case of high dose CP toxicity. Potential to ameliorate the CP evoked toxicity as well as to improve the chemotherapeutic effect could have beneficial implications for patients undergoing chemotherapy with cisplatin.

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