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Research Article

EFFECT OF DECOCTION OF ROOT BARK OF *BERBERIS ARISTATA* AGAINST CISPLATIN-INDUCED NEPHROTOXICITY IN RATS

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ABSTRACT

The formulation prepared only with the *Berberis aristata* is prescribed by ayurvedic physicians to treat various diseases. The purpose of present study is to evaluate its nephroprotector activity against cisplatin-induced renal toxicity in rats. The plant decoction is administered orally (500 and 1000mg/kg b.w.). The effect of decoction of root bark of *B. aristata* is examined in terms of blood urea nitrogen, serum creatinine, urinary protein, urine to serum creatinine ratio, lipid peroxidation and histological aspects of kidney. Cisplatin-induced significant elevation of blood urea nitrogen, serum creatinine, urinary protein excretion, lipid peroxidation and reduced the levels of urine to serum creatinine ratio and glutathione. In curative regimen, decoction of root bark of plant significantly reversed the all the effects induced by cisplatin in dose dependent manner. The plant decoction also effectively protected from cisplatin-induced effects in prophylactic regimen. The above results are substantiated by histological studies. In conclusion, the present study provides the corroborative scientific evidence for the folklore use of *B. aristata* in urinary troubles.

Key words: *Berberis aristata*, Cisplatin, Lipid peroxidation, Nephroprotector activity

INTRODUCTION

Nephrotoxicity can be defined as renal disease or dysfunction that arises as a direct or indirect result of exposure to medicines and industrial or environmental chemicals. Over the past several years, the number of persons suffering from renal problems is increasing. The reasons are: exposure to medicines, industrial/environmental chemicals, age, pre-renal disease etc. Drugs such as cisplatin, gentamicin, paracetamol and adriamicine induce nephrotoxicity. Cisplatin (Cis-diammine dichloro platinum-II) is a potent anticancer agent used to treat various solid tumours such as head, neck and testicular carcinoma [1-3]. A Number of studies reported that cisplatin clinical use is limited by its nephrotoxicity [4-6].Several mechanisms have been suggested for cisplatin-induced renal toxicity i.e., apoptosis, inflammatory mechanism and generation of reactive oxygen species [7]. Many compounds (synthetic and natural) are screened for nephroprotector activity against cisplatin-induced nephrotoxicity, in these studies treatment with plant extracts is quite encouraging [8-12]. *Berberis aristata* (family Berberidaceae) is one such plant widely used in ayurveda to treat urinary problems [13] with no scientific evidence. Hence, present is planned to evaluate nephroprotector activity of decoction of root bark of *Berberis aristata* against cisplatin-induced nephrotoxicity.

MATERIALS AND METHODS

Plant Material

The dried stem bark of *Berberis aristata,* which was used for present study was procured from Sri Srinivasa Ayurvedic Pharmacy, Tirumala Tirupati Devasthanams, Tirupati, Andhra Pradesh, and was powdered in Wiley mill.

Preparation of Decoction

To 250 g of the *Berberis aristata* powder,1100 ml of water was added and boiled on water bath, for half an hour, kept overnight at room temperature (25±2°C) and filtered. The filtrate was concentrated to 600 ml and the decoction (63 mg/ml) was used for the present study.

EXPERIMENTAL

Animals

The study was performed on Wistar strain albino rats of either sex (120 days, weighing 150-200g). They were maintained in standard conditions, diet (Gold Mohur pellets, Bangalore) and water was given *ad libitum.* The study was conducted after obtaining Institutional ethical committee clearance.

Acute toxicity and gross behavioral studies

Animals were divided into 6 groups, each group containing 6 animals. The first group was control group and the remaining groups were experimental groups which received different doses (100, 300, 600, 1000 and 3000 mg/kg) of decoction of the root bark of *B. aristata* to study gross behavioral responses like vocalization on touch, loco motor activity, palpebiral reflex, autonomic responses such as tremors, convulsion, salivation, diarrhea, sleep, coma and observed continuously for 2 h and intermittently once every 2 h and then monitored for any mortality for the following 14 days.

Assessment of renal function [14]

Blood urea nitrogen (BUN: Di acetyl monoxime method) , serum creatinine (SC: Alkaline picrate method) and serum total proteins (S_{TP}: Biuret method) were estimated by using commercial kits. Urine was collected on day 15 (prophylactic regimen), 5 and 16 (curative regimen) for 6 h (initiated at 8 AM) by keeping the animals in individual metabolic cages and was analyzed for creatinine and protein (Up: sulphosalicylic acid method).Creatinine clearance was calculated by using following formula: Creatinine clearance = urinary creatinine X urinary volume h^{-1}/ serum creatinine

Renal toxicity

Animals were divided into 7 groups (n=6) and were put on treatment schedule given in Table-1. To induce nephrotoxicity in rats, cisplatin dose selected was 6 mg/kg (intraperitoneally, single dose). The decoction (63 mg/kg) was administered orally by gastric intubation.

Lipid peroxidation in kidney

Lipid peroxidation was evaluated as malondialdehyde (MDA) production as described by Heath and Backer [15]. The animals were sacrificed by decapitation on day 15 or 16. The kidneys were dissected out, immediately placed in ice cold saline to prevent contamination with blood and they were pressed on blotted paper, weighed and homogenized in 1.5% KCl with the help of Teflon homogenizer. To 1 ml of homogenate, 2.5 ml of trichloroaceticacid (TCA, 20%) was added and centrifuged at 3500 rpm for 10 min. The resulting pellet was dissolved in 2.5 ml of 0.05 M H_2SO_4 and then 3 ml of thiobarbituric acid was added and incubated at 37°C for 30 minutes. The contents were then extracted into 4ml of n-butanol and the absorbance was measured spectrophotometrically at 530 nm.

Table 1: Treatment schedule for evaluating the nephroprotector activity of decoction of root bark of *B. aristata*

Group	Treatment	Day of biochemical estimations	Purpose
I	Normal animals- received only vehicle (distilled water)	5^{th},15^{th} &16^{th}	Normal Control
II	curative control- Cisplatin on day1, Vehicle 10 days (6^{th} to15^{th} day).	5^{th} &16^{th}	To serve as control for groups III_a& III_b
III_a	Curative -Cisplatin on day 1,Plant Decoction (500 mg/kg) from day 6 to15.	5^{th} &16^{th}	To assess curative effect of *B. aristata*
III_b	Curative- Cisplatin on day 1, Plant Decoction (1000mg/kg) from day 6 to15.	5^{th} &16^{th}	To assess curative effect of *B. aristata*
IV	Prophylactic control -Vehicle from day 1 to10, Cisplatin on day 11.	15^{th}	To serve as prophylactic- control for group V
V	Plant Decoction (1000 mg/kg) from day 1 to10, Cisplatin on day 11.	15^{th}	To assess prophylactic effect *B. aristata*
VI	Plant Decoction (1000 mg/kg) from day 1 to10.	11^{th}	To observe effect of plant on Kidneys

HISTOLOGICAL STUDIES

Two animals from each group were sacrificed on day fifteen or sixteen and kidneys were isolated. The kidney sections were stained with hematoxylin and eosin and observed under light microscope.

STATISTICAL ANALYSIS

The results are expressed as mean±SEM and the data was analysed by one way analysis of variance followed by post hoc Student-Keuls test using SPSS computer software for *in vivo* studies. Statistical significance was set at P≤ 0.05.

RESULTS

Acute toxicity and gross behavioral studies

The decoction of root bark of *B. aristata* was found to be safe since no animal died even at the maximum dose of 3000 mg/kg body weight. The animals did not show any gross behavioral changes.

Pharmacological studies

Treatment of decoction of root bark of *B. aristata* for 10 days (VI) did not caused any significant changes in serum markers and renal functional parameters level when compared with normal control animals. Hence, decoction of *B. aristata* did not show any deteriorative effects on kidney. To assess curative activity, the data obtained from the treated groups (III_a, III_b) was compared with respective curative control group (II). Similarly, prophylactic activity (V) was assessed in comparison with prophylactic control (IV).

Effect of decoction on serum markers level

Table 2 lists the effect of decoction of *B. aristata* on cisplatin-induced nephrotoxicity. Intraperitoneal administration of cisplatin at 6 mg / kg. caused significant elevation of BUN, SC and S_{TP} in group II and IV animals, when compared to normal control animals (I).Treatment with decoction of *B. aristata* for 10 days caused a significant reduction in the BUN, SC and S_{TP} in curative groups (III_a and III_b) when compared to its control group (II). The decrease in serum markers level was more in animals treated with 1000mg/kg Bd.wt decoction than with 500mg/kg, indicating a dose dependent effect of decoction of root bark of *B. aristata.* In preventive regimen, treatment with decoction of root bark of *B. aristata* for 10 days, there is a significant reduction of serum markers level when compared to the preventive-control group (IV).

Table 2: Effect of *Berberis aristata* decoction on cisplatin-induced nephrotoxicity

Group	BUN (mg/dl)	SC(mg/dl)	S_{TP}(g/dl)	LPO(nmol/mg tissue)
I	23.7±3.8	0.7±0.1	6.1±0.2	0.44±0.08
II	62.2±3.2[a]	1.0±0.2 [a]	7.9±0.9 [a]	1.11±0.15 [a]
III_a	45.8±1.7[b]	0.9±0.1 [b]	7.0±0.1 [b]	1.12±0.20 [b]
III_b	37.9±8.5[a,b]	0.8±0.1 [a,b]	6.6±0.5 [a,b]	0.53±0.09 [a,b]
IV	100.8±2.3[a]	2.4±0.4 [a]	9.7±0.7 [a]	0.71±0.29 [a]
V	92.3±5.8[c]	1.9±0.5 [c]	8.5±0.4 [c]	0.62±0.03 [c]

Values are expressed as mean±SEM of six observations,*[a]P<0.05 compared with normal control. *[b]P<0.05 compared with curative control. *[c]P<0.05 compared with prophylactic control (one-way ANOVA followed by student's Newman–Keuls post hoc test)

Effect of decoction on renal functional parameters

The deterioration of renal functions induced by cisplatin and the effect of oral administration of the decoction of root bark of *B. aristata* are given in Table 3. Administration of cisplatin caused significant reduction in the urine to serum creatinine ratio (Ucr / Scr), creatinine clearance and increased excretion of urinary protein in the curative-control (II) and preventive-control (IV) groups respectively, when compard to the normal control group (I). In curative regimen, treatment with decoction in group IIIa and III_b animals significantly increased the urine to serum creatinine ratio, creatinine clearance and reversed the elevated levels of total protein excretion caused by cisplatin when compared to respective control (II). Animals pretreated with decoction for 10 days (V) showed significant protection against cisplatin-induced effects.

Lipid peroxidation (LPO)

Single intraperitoneal administration of cisplatin at 6 mg / kg caused significant increase in the levels of MDA in curative (II) and preventive control (IV) groups when compared to the normal group (I), indicates increased lipid peroxidation in group II and group IV animals. On treatment with fresh decoction in groups IIIa and IIIb (curative groups) a significant decrease in the levels of MDA was observed when compared to the curative control group and prophylactic treatment with decoction in group V (preventive group) also significantly decreased the MDA levels when compared to the prophylactic control group.

Histological Studies

Conventional histological examination using light microscope on hematoxylin-eosin- stained sections showed striking differences in the cisplatin- treated rat kidney (group II and IV) when compared to sections belongs to normal rat kidney (group I). This histological abnormality includes marked congestion of the glomeruli with glomerular atrophy, degeneration of tubular epithelial cells with cast (Figures -2 and 4). Treatment with *B. aristata* decoction in curative group (group IIIb) regenerative changes in tubular cells and glomeruli was observed indicating marked protection against renal injury caused by cisplatin (Figure - 3). In preventive group moderate regenerative changes were observed (Figure - 5).

Table 3: Effect of Cisplatin and *Berberis aristata* decoction on renal functions

Treatment	U_{cr}/S_{cr}	Cl_{cr} (ml/hr/100g Bd.wt.)	Up (mg/24 hr)
I	17.7±0.5	19.0±0.2	6.0±0.3
II	7.6±0.2 [a]	13.7±0.6 [a]	18.9±1.3 [a]
III_a	11.8±0.7 [b]	14.5±0.8 [b]	12.7±0.8 [b]
III_b	14.1±1.4 [a,b]	17.7±0.5 [a,b]	7.8±0.4 [a,b]
IV	7.6±0.2 [a]	10.0±0.6 [a]	18.5±1.4 [a]
V	9.6±0.5 [c]	13.3±0.8 [c]	16.5±1.1 [c]

Values are expressed as mean±SEM of six observations. *[a]P<0.05 compared with normal control. *[b]P<0.05 compared with curative control *[c]P<0.05 compared with prophylactic control (one-way ANOVA followed by student's Newman–Keuls post hoc test)

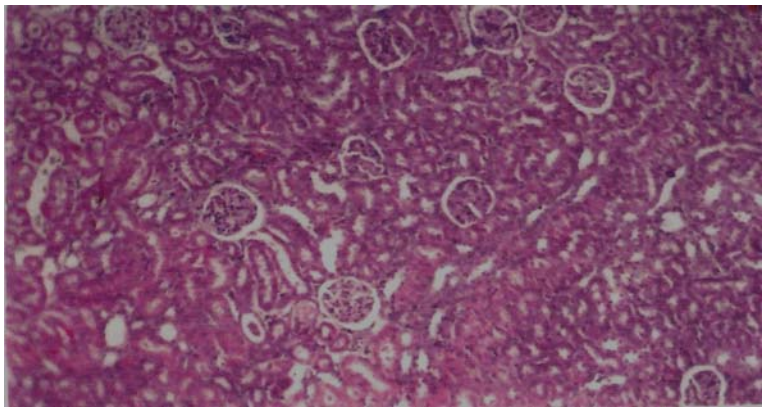

Fig. 1: Section of normal rat kidney showing normal organization of tubular epithelial cells and glomeruli (X-70)

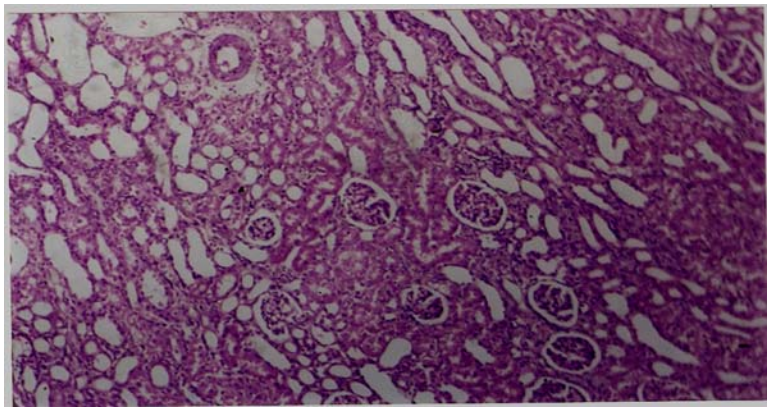

Fig. 2: Section of rat kidney treated with cisplatin (curative control) showing congestion in glomeruli, glomerular atrophy, disappearance of nuclei in tubular cells (X-70)

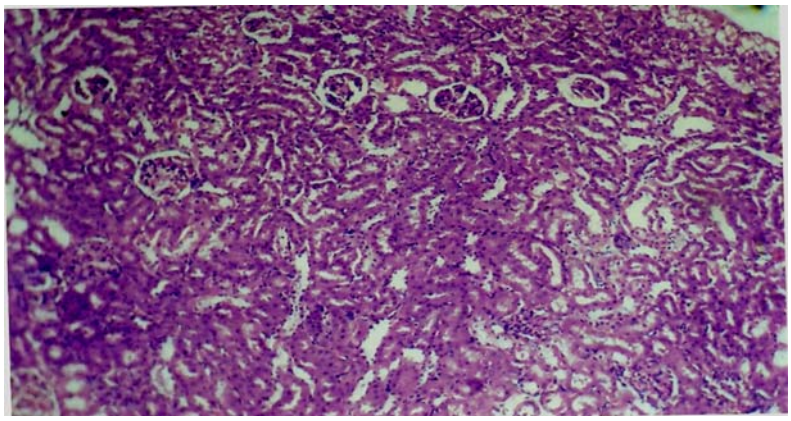

Figure 3: Section of rat kidney treated with decoction of *B. aristata* [curative activity (500 mg/kg)] showing moderate regenerative changes but still presence of congestion in glomeruli, glomerular atrophy (X-70)

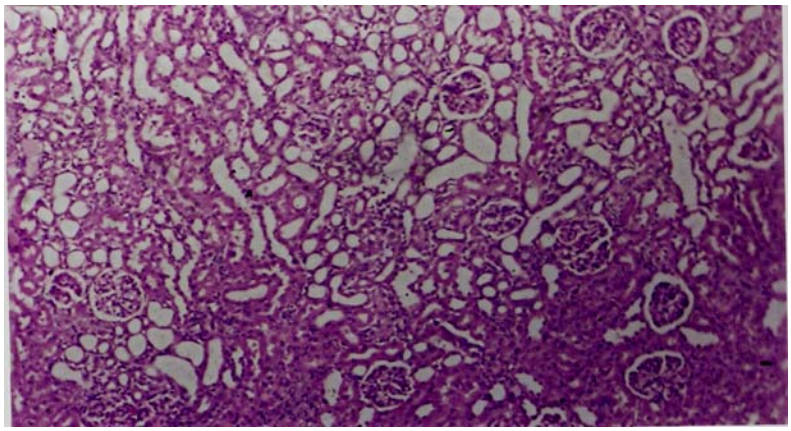

Fig. 4: Section of rat kidney treated with decoction of *B. aristata* [curative activity 1000 mg/kg)] showing regenerative changes in tubules and glomeruli (X-70)

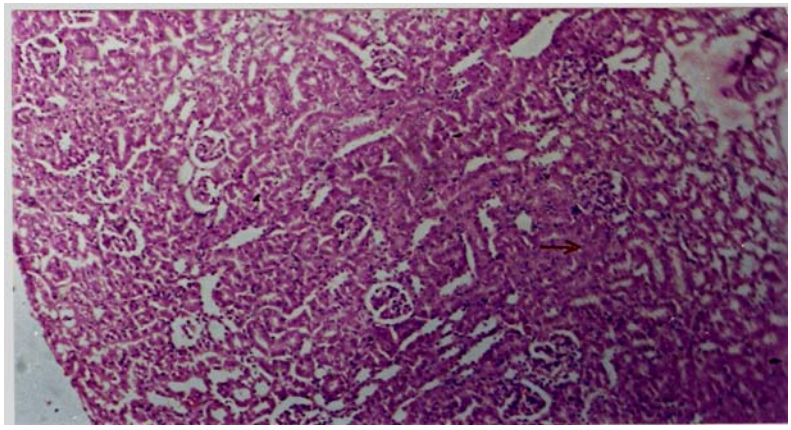

Fig. 5: Section of rat kidney treated with cisplatin (prophylactic control) showing severe congestion in glomeruli, presence of cast cells, glomerular atrophy (X-70)

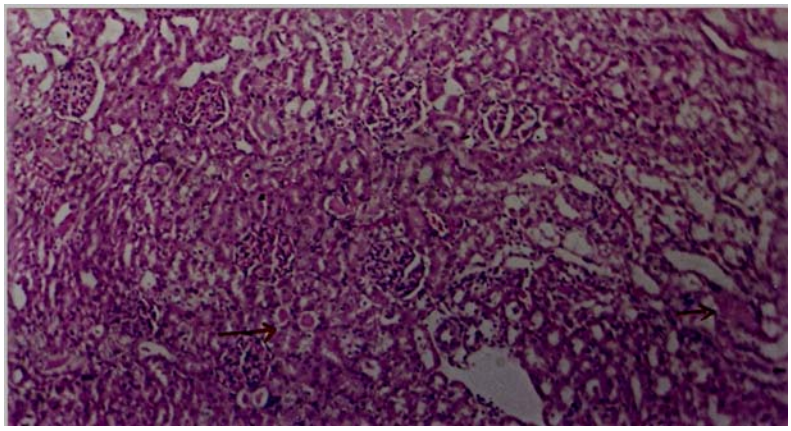

Fig. 6: Section of rat kidney treated with decoction of *B. aristata* [prophylactic activity (1000 mg/kg)] showing presence of cast cells and inter-tubular haemorrhage (X-70)

DISCUSSION

In recent years due to various reasons the number of persons suffering from renal diseases are gradually increasing, exposure to certain drugs is one of the cause for renal toxicity. Till today there are no drugs available which could effectively prevent the incidence/ development or cure the renal damage caused by various agents such as some drugs, industrial/environmental chemicals. Cisplatin has broad activity as an antineoplastic agent, and especially useful in the treatment of head, neck, bladder, esophagus and lung cancer [1, 2] but clinical use of cisplatin is limited by its nephrotoxicity. Number of reports has reported that a single intraperitonial administration of cisplatin induced nephrotoxicity [13, 14]. Although the mechanism of cisplatin nephrotoxicity is not clear, but Several mechanisms have been suggested for cisplatin-induced renal toxicity i.e., apoptosis [4], inflammatory mechanism [5] and generation of reactive oxygen species [6,7]. Recent reports reveled that plants containing antioxidant principles were reported to possess protection against

cisplatin- induced nephrotoxicity [11, 12]. *B. aristata* is one such plant widely used in ayurveda to treat various diseases [13].

The present study results of our study confirmed that cisplatin at 6 mg/kg produces significant nephrotoxicity as indicated by increase in blood urea nitrogen, serum creatinine, urinary protein excretion and MDA levels in kidney. Further it was characterized by significant reduction in the urine to serum creatinine ratio (Ucr/Scr), creatinine clearance. In curative regimen, the decoction exhibited marked protection against the renal and functional impairment induced by cisplatin at both the doses tested. However, the protection is more significant at the higher dose (1000 mg/kg p.o.). This was characterized by changes in serum markers level and urinary functional parameters.

Induction of nephrotoxicity by cisplatin is assumed to be a rapid process involving reaction with proteins in the renal tubules [16]. Because this renal damage occurs in the first hour after administration, it is important that the protective agent be present at sufficient concentrations in renal tissue before the damage occurs [17, 18]. This is the rationale behind the prophylactic treatment of decoction of selected plant in advance of cisplatin. In prophylactic regimen, pretreatment with the decoction of root bark of *B. aristata* at 1000 mg/kg (p.o.), significantly reversed the effects that are caused by cisplatin.

The reaction of lipid peroxides with TBA has been widely adopted as a sensitive assay method for lipid peroxidation in animal tissues. Previous reports suggest cisplatin induced nephrotoxicity is by initiation of lipid peroxidation [19]. Earlier reports suggesting that cisplatin exerts its nephrotoxic effects by generation of free radicals [20, 21]. In present lipid peroxidation studies, animals pretreated with plant decoction showed moderate protection against cisplatin-induced elevated levels of MDA. In curative regimen, animals which received 1000 mg/kg dose showed good protection on cisplatin-induced elevated levels of MDA. Histological Studies also substantiated the above results.

The root bark of *B. aristata* was reported to contain alkaloids such as berberine, aromoline, oxyberberine, karachine and taxilamine [22-25]. Berberine is a chief alkaloid present in root bark of *B. aristata* and wide range of pharmacological and biological activities including anti inflammatory,anti microbial, anti tumor and anti diabetic activities [26-29]. The lipoxygenase inhibition and antioxidant properties of berberine have also been reported by Misik et al., [30]. Hence, the antioxidant property of berberine may be partially responsible for nephroprotector activity exerted by *B. aristata.*

In conclusion, *B. aristata* supplementation during cisplatin therapy reduces the risk of cisplatin-induced nephrotoxicity and protection may be attributed to a decrease in lipid peroxidation formation. Further, the present study provides the corroborative scientific evidence for the folklore use of *B. aristata* in urinary troubles.

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