

Protective Effect of Root Extracts of *Bauhinia Variegata* Linn against Cisplatin-Induced Nephrotoxicity in Rats

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Abstract

In this study, we aimed to investigate to possible protective effect of *Bauhinia variegata* Linn. on Cisplatin induced nephrotoxicity. Experimental were carried out in male Wistar rats weighing 200-250 g. Cisplatin (5 mg/kg body weigh i.p.) and Cisplatin together with ethanolic and aqueous root extracts of *Bauhinia variegata* (BVE) at 400 mg/kg b.w. respectively were administrated for 7 days. The animals were scarified 24 h after the last injection. Serum creatinine, serum urea, urine creatinine and blood urea nitrogen (BUN) were determined before sacrificed. Kidneys were collected for determination of antioxidant status and for histopathological studies and fixed in 10% buffered formalin solution. Tissue sample were stored at -70 °C in liquid nitrogen for determination of glutathione (GSH), glutathione-S-transferase (GST), malondialdehyde (MDA) and catalase (CAT). Glutathione assay was determined by the method of Beutler, *et al.* [1]. GST amounts were measured by the method of Habig *et al.* [2]. Catalase activity was tested by Aebi's method and MDA was determined according to Thayer's method [3,4]. Significantly decrease in serum creatinine, serum urea, urine creatinin levels in extract treated groups which were elevated by Cisplatin. These results were confirmed by histopathological study which demonstrated that root extract of *Bauhinia variegata* has a protective effect against Cisplatin induced nephrotoxicity, lipid peroxidation and cellular damage in rats.

Keywords: Nephrotoxicity; Lipid Peroxidation; *Bauhinia vereigata* Linn.

Introduction

Cisplatin (cis-diammino dichloro platinum II) is currently one of the most important chemotherapeutic agents used in the treatment of a wide range of solid tumors– head, neck, ovarian and lung cancers. However, the clinical usefulness of this drug is limited due to nephrotoxicity induction, a side effect that may be produced in various animal models [5]. Cisplatin preferentially accumulate in cells of the S3 segment of the renal proximal tubules and is toxified to form a reactive metabolite intra cellularly by hydration. The peroxidation of membrane lipids may account for its nephrotoxicity. Available evidence suggests that cisplatin exerts its nephrotoxic effects by the generation of free radicals.

Cisplatin gets accumulated in the tubular epithelial cells of proximal kidney tubule, causing nephrotoxicity, characterized by mitochondrial destruction of intracellular organelles, cellular necrosis, loss of microvilli, alteration in the number and size of the lysosomes and mitochondrial vacuolization, followed by functional alteration including inhibition of protein synthesis, GSH depletion, lipid peroxidation and mitochondrial damage [6].

A large number of studies have reported the beneficial effects of a variety of antioxidants in cisplatin induced nephrotoxicity. The plant *Bauhinia variegata* Linn. (*Caesalpinaceae*) commonly known as Mountain Ebony is a medium-sized, deciduous tree, found throughout India. It has been used in dyspepsia, bronchitis, leprosy, ulcer, to prevent obesity, as an astringent, tonic and anthelmintic [7]. The stem contains -sitosterol, lupeol, kaempferol-3-glucoside and 5,7-dihydroxy and 5,7-dimethoxy flavanone-4-O--L-rhamnopyranosyl--D-glucopyranosides. Flowers contain cyanidine-3-glucoside, malvidin-3-glucoside, malvidin-3-diglucoside, and peonidin 3-diglucoside, kaempferol-3-galactoside and kaempferol-3-rhamnoglucoside. Five flavonoids isolated from the different parts of *Bauhinia variegata* has been identified as quercetin, rutin, apigenin and apigenin 7-O-glucoside. Phytochemical analysis of the root bark of *Bauhinia variegata* Linn was reported to contain a new flavanone: (2S)-5, 7-dimethoxy-3'-4'-methylene dioxyflavanone (1) and a new dihydrobenzoxepin 5, 6-dihydro-1,7dihydroxy-3,4-dimethoxy-2-methylidibenz (b,f) oxepin [8,9].

Bauhinia variegata Linn. stem is reported to have antitumor, antimicrobial, anti-inflammatory, hepatoprotective, anti-hyperlipidemic and immunomodulatory activities [10-15]. The present study is aimed to investigate the possible effect of ethanolic and aqueous extracts of nephroprotective effect of *Bauhinia variegata* Linn. in cisplatin induced nephrotoxicity in rats.

Material and Methods

Plant material

The root of *Bauhinia variegata* Linn. Was procured and authenticated by Shri A. V. Bhatt, survey officer, Regional Research Institute (Ay.), Bangalore, Karnataka (India). A voucher specimen of same has been deposited (voucher specimen no. RRCBI MCW 79/4).

Preparation of the root extract

The authenticated root was shade dried and powdered coarsely. Extraction was done according to standard procedures using analytical grade solvents. The powdered drug was defatted by extracting with pet-ether (60-80 °C). Coarse powder of the root (1 Kg) was soxhlet extracted with 90% ethanol [16]. The aqueous extract was prepared by the process of maceration. The extracts obtained were concentrated under reduced pressure to yield ethanolic (4.2%) and the aqueous extracts (2.4%).

Animals

Albino male Wistar rats weighing between 150 and 200 gm were procured from registered breeders (Venkateshwara Enterprises, Bangalore). The animals were housed under standard conditions of temperature (25±2 °C) and relative humidity (30-70%) with a 12:12 light-dark cycle. The animals were fed with standard pellet diet and water *ad libitum*. Approval of the Institutional Animals Ethics Committee (IAEC/KLECP/ BNG/06/2009) of K.L.E.Society's College of Pharmacy, Bangalore was taken for conducting nephroprotective activity.

Acute toxicity studies

Acute toxicity studies for aqueous and ethanolic extracts of *Bauhinia variegata* Linn. Were conducted as per OECD guidelines 423 using albino Wistar rats [17]. Each animal was administered the aqueous solution of the extract by oral route. The animals were observed for any changes continuously for the first 2h and upto 24h for mortality.

Animal and Experimental Protocol

Albino rats (150-200 gm) of either sex were used for study. Animals were divided into six groups, each group containing six animals. Study was carried out for eight days and treatment was given for seven days [18,19].

Group I: Served as control group and received distilled water p.o. for seven days.

Group II: Served as cisplatin (Biochem pharmaceutical Industries Ltd.) group.

Groups III: Received 400 mg/kg b.w. of BVA (p.o.) for 7 days.

Groups IV: Received 400 mg/kg b.w. of BVE (p.o.) for 7 days.

On day 2 cisplatin 5 mg/kg b.w. was administered i.p. to all the animals of groups II, III and IV. After dosing on the day 7, individual rats were placed in separate metabolic cages for 24h for urine collection to determine urine output and urine creatinine content. Blood samples were collected through retro orbital method and the serum was rapidly separated and processed for determination of blood urea nitrogen (BUN) and serum creatinine using auto analyser (Span Diagnostic kits).

Sample collection and Biochemical Assays

The animal in all groups were decapitated 24 h after the last application. Blood samples were collected through retro orbital method into the tubes containing anticoagulant (2% solution oxalate) agent. The blood samples were centrifuged at 200 xg for 5 min at +4 °C to separate the plasma and one kidney tissues were removed immediately from each group and stored at -20 °C until analysed. The homogenization of tissues was carried out in Teflon-glass homogenizer with a buffer containing 1.15% KCl to obtain 1:10 (w/v) whole homogenate. Homogenate were centrifuged at 18000 xg (+4 °C) for 15 min to determine MDA, GSH concentration. All kits were purchased Span Diagnostic kits. The supernatant was used as the source of experimental product. Glutathione (GSH) assay was determined by the method of, with 5,5 dithio bis nito benzoic acid as product. Glutathione-S-transferase (GST) amount were measured [1,2]. Rat kidney homogenate malondialdehyde (MDA) levels were measured by Thayer's method (1985) [4]. All the measurements were carried out by a spectrophotometry (Shimadzu UV-VIS spectrophotometer, Japan).

Histopathological examination

Another kidney was excised after sacrifice. The tissue were fixed in 10% formaline, embedded in paraffin, sectioned at 5 µm and stained with haematoxylin-eosin. Light microscopy was used to evaluate tubular necrosis, glomerular congestion, inflammatory cell and peritubular congestion which were graded as follows:

Mild (+): only single cell necrosis and slight degenerative changes.

Moderate (++): tubular necrosis at different foci throughout the cortex.

Severe (+++): extensive and marked tubular necrosis throughout the cortex.

Statistical analysis

The data are expressed as mean \pm SD. Results were analysed statistically by one-way analysis of variance (ANOVA) followed by Dunnett. P- value <0.05 was regarded as statistically significant.

Results

Acute toxicity study

There was no change in normal behavioral pattern of animals and no sign and symptoms of toxicity were observed during the observations which was done continuously for the first two hours and then observed up to twenty four hours for mortality. The extracts were safe up to a maximum dose of 2000 mg/ kg b.w. The biological evaluation was carried out at doses of 400 mg/kg b.w.

Effect of Cisplatin and *Bauhinia variegata* treatment on level of plasma creatinine, urea, urine creatinine, Na⁺, K⁺ and body weight

First set of experiments examined the effects of Cisplatin on kidney function. Table 1 showed the effect of treatment on of plasma creatinine, urea, urine creatinine, Na⁺ and K⁺ levels.

Group	Treatment	Serum creatinine (mg/dl)	Serum urea (mEq/l)	Urine creatinine (mg/dl)	Na ⁺ (mEq/l)	K ⁺ (mEq/l)	Body weight (%change)
I	Control	0.71 \pm 0.73	43.17 \pm 0.15	96.71 \pm 3.54	140.08 \pm 1.16	5.25 \pm 0.35	3.23 \pm 0.27
II	Cisplatin	3.11 \pm 0.67 ^{****a}	199.6 \pm 1.78 ^{****a}	255.8 \pm 5.56 ^{****a}	142 \pm 1.83	5.49 \pm 0.30	-17.4 \pm 1.32 ^{****a}
IV	Cisplatin+ BVA 400	0.83 \pm 0.12 ^{****b}	76.34 \pm 1.29 ^{****b}	115.8 \pm 2.61 ^{****b}	139.3 \pm 0.92	5.35 \pm 0.32	-7.41 \pm 1.7 ^{****b}
VI	Cisplatin+ BVE 400	0.72 \pm 0.12 ^{****b}	71.47 \pm 1.15 ^{****b}	106.8 \pm 3.63 ^{****b}	138.37 \pm 1.19	5.37 \pm 0.27	-4.92 \pm 0.49 ^{****b}

n=6, Values are expressed as mean \pm S.D., BVA and BVE 200 and 400 indicate *Bauhinia variegata* aqueous and ethanolic extracts at 200 and 400mg/kg b.w. respectively. * $p<0.05$, ** $P<0.01$, *** $P<0.001$, a- indicates comparison with control group and, b- indicates comparison with cisplatin treated group

Table 1: Nephroprotective activity of ethanolic and aqueous extracts of *Bauhinia variegata* Linn. in cisplatin induced nephrotoxicity in rats

Plasma creatinine, urea and urine creatinine levels ($p<0.001$) were significantly higher at the end of administration of Cisplatin for seven successive days when compared to the control group. However creatinine and urea levels ($p<0.001$) in aqueous and ethanolic extracts of *Bauhinia variegata* treated groups were lower than the treated with Cisplatin alone. Urine creatinine were also significantly ($p<0.001$) decrease in both extracts group when compared with Cisplatin group, Na⁺ and K⁺ levels were unaffected in all groups when compared with the control group. Body weights were decrease in Cisplatin group when compared with control group. All extract treated group were exhibited significantly increase in body weight compared with Cisplatin alone.

Effect of Cisplatin and BVE treatments on kidney MDA, GSH levels and GST, CAT activities

As shown in Table 2, the effect of treatments on kidney levels of MDA and GSH, activities of GST and CAT. Cisplatin treated group had significantly higher level of MDA ($p<0.001$) in kidney tissue, while having significantly lower GST ($P<0.001$) and CAT ($P<0.001$) activities, but had no change in GSH levels when compared with the control group.

Groups	Treatment	GSH (nmol/mg protein)	GST (nmol/mg protein)	CAT (k/mg protein)	MDA (nmol/mg protein)
I	Control	23.06 \pm 0.75	22.58 \pm 0.86	0.65 \pm 0.06	0.023 \pm 0.0027
II	Cisplatin	7.3 \pm 0.62 ^{***}	8.12 \pm 0.52 ^{***}	1.19 \pm 0.03 ^{***}	0.049 \pm 0.0081 ^{***}
III	Cisplatin +BVA 400	18.48 \pm 1.32 [*]	17.0 \pm 1.65	0.60 \pm 0.008	0.030 \pm 0.0053
IV	Cisplatin +BVE 400	19.32 \pm 1.63 [*]	18.0 \pm 1.28	0.63 \pm 0.005	0.027 \pm 0.0029

n=6, Values are expressed as mean \pm S.D., The significance between two groups was determined with Dunnett's multiple comparison test

BVA 400 and BVE 400 indicate *Bauhinia variegata* aqueous and ethanolic extracts at 400mg/kg b.w. respectively

* $p<0.05$, ** $P<0.01$, *** $P<0.001$, statistically significant from control

Table 2: Effect of Cisplatin and its combination with BVL on renal MDA, reduced GSH and GST, CAT activities

Pre-treatment with BVL exhibited decrease in MDA levels ($p<0.05$) when compared with Cisplatin treated group. On the other hand, simultaneously treatment with BVE normalised in kidney tissue MDA levels ($p<0.001$) when compared to Cisplatin treated group. However, simultaneously treatment of both ethanolic and aqueous extracts of BV provided a significant increase in kidney GSH levels ($p<0.001$) and GST activities ($p<0.05$), but a slight increase in pre-treatment with BVE when compared to Cisplatin. CAT activity was higher in simultaneously BVE group ($p<0.05$) than BVE pre-treated ($p<0.005$) and Cisplatin ($p<0.001$) treated group but was lower than the control group.

Effect of Cisplatin and BVE treatments on kidney histology

Histopathological examination of kidney showed severe and extensive damage in Cisplatin treated rats. These changes are summarised in Table 3. Figure 1A indicates a kidney section of a control rat while Figure 1B shows representative image of kidneys of Cisplatin treated rats which have tubular necrosis and edema (Figure 1B). Glomerular congestion. Peritubular congestion, tubular vacuolization, inflammatory cell and tubular necrosis are less severe in both extracts of *Bauhinia variegata* treated rats (Figure 1C and 1D). Ethanolic extract is showed better activity than aqueous extract of *Bauhinia variegata* (Figure 1D).

Histopathological features	Control	Cisplatin	Cisplatin +BVA 400	Cisplatin +BVE 400
Glomerular congestion	-	+++	++	-
Peritubular congestion	-	+++	+	-
Blood vessel congestion	-	+++	++	+
Interstitial edema	-	++	-	+
Inflammatory cells	-	+++	-	-
Mononuclear infiltration		+++	+	-

Haematoxylin and eosin stained skin section were scored as mild (+), moderate (++) an severe (+++) for epidermal and /or re-modelling

Table 3: Histopathological features of the kidneys of rats of different treatment groups in cisplatin induced nephrotoxicity

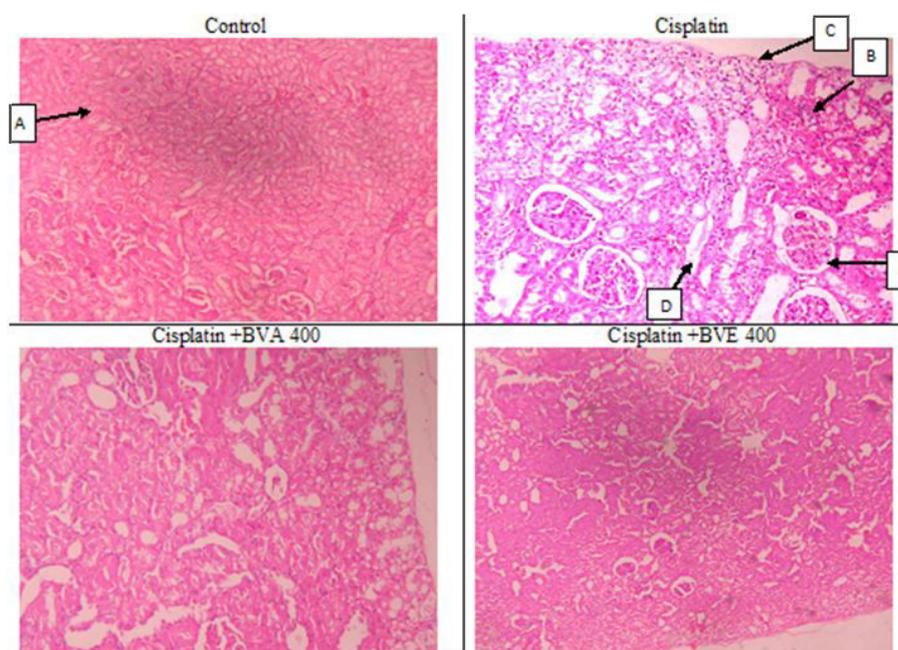


Figure 1: Light microscopy of renal tissue from rats. A- Mononuclear infiltration cells; B- Tubular casts; C- Interstitial edema; D- Blood vessel congestion; E- Glomerular congestion (peritubular congestion, interstitial edema)

Discussion and conclusion

Cisplatin is an effective antineoplastic agent used in the treatment of various solid tumours. Nevertheless, its full clinical utility is limited due to some adverse effects including acute renal failure. Cisplatin preferentially accumulate in cells of the S3 segment of the renal proximal tubules and is toxified to form a reactive metabolite intra cellularly by hydration. The peroxidation of membrane lipids may account for its nephrotoxicity. Available evidence suggests that cisplatin exerts its nephrotoxic effects by the generation of free radicals [19].

The cisplatin induced nephrotoxicity is associated with inhibition of protein synthesis and intracellular GSH and protein-SH depletion, resulting in lipid peroxidation and mitochondrial damage. The production oxidative stress in kidney has been implicated in the pathogenesis of cisplatin-induced renal injury. It has been shown that superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($\cdot OH$) are involved in cisplatin-induced nephrotoxicity. In addition, it has been found that renal lipid peroxidation is increased and glutathione is decreased in this experimental model [20].

It is reported that cisplatin induced nephrotoxicity is by initiation of lipid peroxidation and depletion of cellular thiols compounds. Cisplatin inhibits activities of antioxidant enzymes (SOD, CAT, and GPX) in rat kidneys, suggesting that cisplatin cytotoxicity results from generation of reactive oxygen species (ROS). It has been suggested that cisplatin is able to generate ROS and that it inhibits the activities of antioxidants enzymes in renal tissues. Direct interaction of cisplatin with DNA generates superoxide anion in a cell free [19,21].

The involvement of oxidative stress is further supported by the fact the antioxidants such as vitamin C and E, melathion and selenium and cadmium prevent cisplatin-induced nephrotoxicity. Interestingly, over expression of heme oxygenase-1 ameliorates and heme oxygenase-1 deficiency aggravated renal damage induced by cisplatin, supporting additionally the involvement of oxidant stress in this experimental model [22].

McCall and Feri (1999) reported that dietary supplementation of β - carotene together with vitamin E reduced plasma MDA levels suggesting that they protect against lipid peroxidation, in contrast β -carotene alone had no effect on lipid peroxidation [23]. Preventive effect of the carotenoid are evidenced mainly at doses, which, therefore, many enhance its pro-oxidant character. A lack of adequate defence and/or a chronic oxidative stress result in increased of pro-oxidant effects by carotenoids [24]. The administration of carotenoid supplements such as BVL is associated with protective effects.

In the present study, cisplatin-induced nephrotoxicity was established by administration of single dose of cisplatin (5mg/kg) i.p. on day 2 of the experiment. The toxicity was characterized by increase in serum creatinine, serum urea, BUN and urine creatinine levels and tubular necrosis. Significant ($p < 0.001$) elevations in the levels of serum creatinine, serum urea, urine creatinine and blood urea nitrogen were produced in the cisplatin group when compared to the control group. However, these changes were reversed by treatment with single daily oral dose 400 mg/kg b.w. of BVA and BVE for 7 days. There was a significant ($p < 0.001$) decrease in serum creatinine, urine creatinine, BUN and serum urea levels in all the animals treated with BVA 400 and BVE 400 extracts when compared with cisplatin group.

Decrease in kidney MDA levels and increase in activity in activities of GST and CAT were observed in kidney tissues of rats especially in simultaneous BVE treatment compared to Cisplatin treated group. Whereas, GSH level were not affected by treatment with Cisplatin and BVE pre-treatment (Table 2). Also significant are the histopathological finding of the present study which are in accordance with the histological lesion recorded in cisplatin induced nephrotoxicity. Extracts of BVL as an antioxidant, inhibit lipid peroxidation and prevent tubular cell injury and also prevent cell damage such as tubular vacuolization, glomerular congestion and interstitial edema caused by cisplatin treatment. The reduction in body weight following cisplatin administration may possibly be due to the injury renal tubules and the subsequent loss of the tubular cells to reabsorb water, leading to dehydration and loss of body weight. Treatment with extracts antagonized the reduction in body weight significantly, but not completely. The alleviation of cisplatin-induced body weight reduction is a reflection of the general palliative effect of antioxidant effect of extracts on the nephrotoxicity.

Antioxidants are compounds that act as inhibitors of the oxidation process and are found to inhibit oxidant chain reaction at small concentration and thereby eliminate the threat of pathological processes. Phenolic compounds present in medicinal plants have been reported to possess powerful antioxidants activity [14].

Flavonoids are major class of phenolic compounds and are found to have a potential role in prevention of various diseases through their antioxidant activity. Flavonoids particularly quercetin isolated from other nephroprotective medicinal plants has been reported of inhibit xenobiotics-induced nephrotoxicity in experimental animal models, due to their antioxidant or free radicals scavenging effects.

Flavonoids and quercetin in particular are potent antioxidants and are known to modulate the activities of various enzyme systems due to their interaction with various biomolecules. They show antiatherogenic and anticarcinogenic activities by blocking LDL oxidation and inhibition of processes of bioactivation of carcinogens. Cytoprotective effect of quercetin may also be due to ability to interact with and penetrate the lipid bilayers. Quercetin decreases the lipid peroxide formation, restoration of glutathione status and the activities of antioxidant enzymes during cisplatin-induced nephrotoxicity [25,26].

The ethanolic and aqueous extracts of root of *Bauhinia variegata* Linn. were able to reverse the elevated levels of serum creatinine, serum urea, BUN and urine creatinine induced by cisplatin as well as by gentamicin. This suggests that the extracts exert nephroprotective activity in both cisplatin as well as Gentamicin induced nephrotoxicity models. They could also ameliorate the histological damage induced by the nephrotoxicants. Both these models produce nephrotoxicity through free radicals apart from other mechanisms. Scavenging of free radicals prevent cell injury and necrosis. Gentamicin induced nephrotoxicity is reported to be reversed by antioxidants.

The aqueous and ethanolic extracts of root of *Bauhinia variegata* Linn. has been reported to have significant antioxidant and anti-inflammatory activities. It is also reported to have flavonoids like quercetin, rutin, apigenin along with sterols, saponins and tannins. The decrease in the expression of TNF receptor associated factor in kidney tissue after rutin supplementation in cisplatin treated rats suggest that rutin may protect against CP induced nephrotoxicity by regulating apoptosis pathway [27]. Quercetin also has significant cytoprotective effect in cisplatin induced renal tubular damage *in-vivo* in rats [28]. Signs of inflammation as evident by inflammatory cells have been noticed in the kidney histopathological slides in the cisplatin treated animals, which was reduced in the treated group animals. This indicates that the ethanolic and aqueous extracts of *Bauhinia variegata* Linn. might have produced nephroprotective activity by the virtue of their antioxidant and anti-inflammatory activities. Antioxidants are known to produce cytoprotective activity. The active constituents present in the root of *Bauhinia variegata* Linn may be responsible for the nephroprotective activity.

In conclusion, this indicates that the ethanolic and aqueous extracts of *Bauhinia variegata* Linn. might have produced nephroprotective activity by the virtue of their antioxidant and anti-inflammatory activities. Antioxidants are known to produce cytoprotective activity. Our finding support that the simultaneous use of BVL extracts may therefore be effective for clinical purpose with antioxidant properties. The active constituents present in the root of *Bauhinia variegata* Linn may be responsible for the nephroprotective activity.

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