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Research Article

PROPHYLACTIC AND CURATIVE EFFECT OF ETHANOLIC EXTRACT OF *BASSIA MALABARICA* BARK AGAINST CISPLATIN INDUCED NEPHROTOXICITY

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ABSTRACT

Objective: The present study was designed to investigate the prophylactic and curative effect of ethanolic extract of *Bassia malabarica* bark (EBBM) against cisplatin (7 mg/Kg single dose *i. p*) induced nephrotoxicity in male albino rats of Wistar strain.

Materials and Methods: After the treatment schedule for 15 days, the extent of nephrotoxicity was quantified using serum samples and isolated kidneys. Serum samples were used to assess levels of protein, creatinine, urea, uric acid and blood urea nitrogen (BUN). The isolated kidneys were used to assess antioxidants such as lipid peroxidation (LPO), reduced glutathione (GSH) and catalase (CAT).

Results: No mortality was observed in acute toxicity studies conducted as per organization for economic co-operation and development guidelines 423 and was found to be safe with no gross behavioral changes up to 2000 mg/Kg body weight (bwt). On administration of cisplatin there was a rise in weight of the kidney, creatinine, urea, uric acid, BUN, LPO and a decrease in bwt, urine volume, total protein, GSH and CAT indicating the role of oxidants to induce nephrotoxicity.

Conclusion: Prophylactic and curative groups were found to ameliorate the cisplatin induced alterations in the kidney in a dose-dependent manner.

Keywords: Antioxidants, *Bassia malabarica,* Cisplatin, Nephrocurative, Nephroprotective

INTRODUCTION

Cisplatin is one of the highly effective antineoplastic drug used to treat solid tumors such as head, neck, lung, bladder, ovarian and testes cancers [1]. However, the clinical usefulness of this drug is limited due to the presence of platinum in the kidney and liver [2,3]. The mechanism of cisplatin nephrotoxicity is still not fully understood, but the importance of reactive oxygen metabolites (ROM) in the cisplatin induced renal cell apoptosis was documented as a major mechanism [4]. Cisplatin induces reactive oxygen species (ROS) in renal epithelial cells primarily by decreasing the activity of antioxidant enzymes and by depleting intracellular concentrations of glutathione (GSH) [5].

Man and his domestic animals have been largely dependent on plants for the essentials of their existence [6]. The demand for herbal drugs or phytomedicines is increasing day by day globally [7]. There is a continuous search for agents which provide nephroprotection against the renal impairment caused by drugs like cisplatin for which allopathy offers no remedial measures. Thus, it was imperative that mankind turns towards alternative systems of medicine for solace [8]. There is a large number of evidence on the chemoprotecting activities of vitamin C, vitamin E, curcumin, selenium, bixin and other dietary components that scavenge free radicals induced by exposure to cisplatin. However, a protective effect of the commonly used antioxidant vitamins such as ascorbic acid, vitamin E remains controversial [9] and protective agents from synthetic sources have been less fruitful [10].

Bassia malabarica belonging to the family sapotaceae is an evergreen tree of 15 m high, bark 6-8 mm thick, dark brown to grey, knotty, deeply fissured and peeling off in rectangular strips, 3 cm long, 2 cm wide. The plant was distributed along the banks of rivers in evergreen and semi-evergreen forests, in the plains of Western Ghats, dry deciduous to moist deciduous forests [11]. Flowers soaked in water are used in the kidney complaints. Fruits are used in rheumatism, consumption, asthma, worms, biliousness and seed oil is used for growth of hair [12], cooking, soap making and for rheumatism [13].

Scientific studies for ethanolic extract of *B. malabarica* bark (EBBM) for its nephroprotective and nephrocurative activity were lacking. Thus, the present study was performed to evaluate the nephroprotective and nephrocurative activity of EBBM bark against cisplatin induced nephrotoxicity.

MATERIALS AND METHOS

Plant material

B. malabarica bark was collected from Tirumala hills and authentified by Dr. Madhavachetti, keeper herbarium, Department of Botany, Sri Venkateswara University, Tirupati. The bark was shade dried at room temperature (25°C).

Drugs and chemicals

Cisplatin was purchased from Cipla and Diagnostic Kits were procured from excel, coral and span diagnostics. All the other chemicals used in the study were of analytical grade procured from Merck and SD Fine Chemicals, Mumbai.

Experimental animals

Adult male albino rats of Wistar strain weighing between 150 and 200 g were used in the present study. The animals were procured from Teena Bio Labs Pvt Ltd (1657/PO/a/12/CPCSEA). The experimental animals were housed in polypropylene cages in a well-ventilated room under hygienic conditions at a temperature of 24±2°C and relative humidity of 70±5%. Animals were exposed to 12 hr day and night cycle. The animals were fed with commercial rat pellet feed and were given water *ad libitum*. All the experimental protocol and procedures were approved by Institutional Animal Ethical Committee (IAEC Reg No. CPCSEA/1657/IAEC/CMRCP/PhD-B/10).

Preparation of the extract
Dried pieces of *B. malabarica* bark was powdered and subjected for successive maceration. The solvents petroleum ether, chloroform, ethyl acetate and ethanol were selected in the order of increasing polarity for maceration. The filtrate obtained after maceration with each solvent was evaporated to dryness under reduced pressure in a rotary vacuum-evaporator at 50°C. The ethanolic extract thus obtained (yield 9.12% w/w) was stored in an airtight container and used for further studies. The extract was subjected to qualitative phytochemical screening for the identification of phytoconstituents [14].

Acute toxicity studies
Acute toxicity study was performed for EBBM according to the acute toxic class method described by organization for economic co-operation and development 423 guideline [15].

Experimental design
The animals were divided into seven groups of eight animals each.

Group I control: The animals received 1% carboxy methyl cellulose (CMC) orally (*p.o*) for 15 days.

Group II negative control: The animals received 1% CMC orally for 15 days, and a single dose of cisplatin 7 mg/Kg body weight (bwt) on day 1 intraperitoneally (*i.p)* to induce nephrotoxicity.

Group III plant control: The animals received 300 mg/Kg bwt of EBBM alone *p.o* for 15 days.

Group IV prophylactic low dose: The animals were pretreated with 150 mg/Kg bwt of EBBM *p.o* for 15 days, and cisplatin was administered on day 11 of 15 days of treatment to induce nephrotoxicity.

Group V prophylactic high dose: The animals were pretreated with 300 mg/Kg bwt of EBBM *p.o* for 15 days and cisplatin was administered on day 11 of 15 days of treatment to induce nephrotoxicity.

Group VI curative low dose: The animals received cisplatin 7 mg/Kg bwt *i.p* on the 1st day of the experiment to induce nephrotoxicity and were administered with EBBM 150 mg/Kg bwt *p.o* for 15 days.

Group VII Curative high dose: The animals received cisplatin 7 mg/Kg bwt *i.p* on the 1st day of the experiment to induce nephrotoxicity and were administered with EBBM 150 mg/Kg bwt *p.o* for 15 days.

After completion of the treatment schedule for 15 days, blood samples were collected by retro orbital puncture and were allowed to clot for 20 minutes. The blood was then centrifuged at 3000 rpm for 10 minutes and the serum obtained was used for estimation of creatinine, urea, uric acid and blood urea nitrogen (BUN). Animals were sacrificed after collecting the blood, and the kidney tissues were homogenized in phosphate buffer pH 7.4. The homogenates obtained were centrifuged at 800 rpm for 5 minutes at 4°C (REMI CM-12) to separate the molecular debris. The supernatant so obtained was centrifuged at 10,500 rpm for 20 minutes at 4°C to get the post-mitochondrial supernatant (PMS) [16].

Biochemical analysis
The PMS obtained was used to estimate enzymatic antioxidants like catalase (CAT) [17] and reduced GSH [18] following standard procedures. The tissue lipid peroxidation (LPO) of kidney was estimated by the method of Niehius and Samuelsson [19]. Serum protein, creatinine, urea, uric acid and BUN were analyzed using commercially available diagnostic kits.

Statistical analysis
Data were analyzed for statistical significance using one-way analysis of variance followed by Dunnet's multiple comparison test, and results were expressed as mean±standard error of the mean (SEM) using Graph Pad Prism Version 5.01.

RESULTS AND DISCUSSION

Phytochemical screening
The preliminary phytochemical screening of EBBM revealed the presence of carbohydrates, glycosides, alkaloids, flavonoids, tannins, phenols, saponins, steroids, carotenoids, and terpenoids.

Acute toxicity studies
The EBBM bark was found to be safe since no mortality was observed even at the maximum dose of 2000 mg/Kg bwt.

Biochemical parameters

Effect of EBBM treatment on bwt, Kidney weight and urine volume against cisplatin induced nephrotoxicity

Negative control group showed a decrease in bwt, urine volume and an increase in kidney weight.

However, all the changes in the negative control group were found to be ameliorated by a significant increase in bwt ($p<0.001$), urine volume ($p<0.001$) and decrease in kidney weight ($p<0.001$) in prophylactic 300 mg/kg bwt when compared to the negative control group as shown in Table 1.

Decrease in the bwt in negative control group treated with cisplatin might be due to diminished appetite and an increase in kidney weight indicates glomerular congestion and tubular necrosis. A significant decrease in urine volume is observed in cisplatin treated negative control group indicating the decrease in glomerular filtration rate. These findings agree with other investigators who reported that the nephrotoxic effect of cisplatin produced marked reduction of the rat's bwt with a significant increase in the relative kidney weight [20-23].

Effect of EBBM treatment on serum parameters against cisplatin induced nephrotoxicity

There was a significant decrease in the levels of bio-chemical parameters like serum creatinine, urea, uric acid and BUN ($p<0.001$) in prophylactic 300 mg/Kg bwt treated group when compared to the negative control group treated with cisplatin 7 mg/kg bwt as shown in Table 2.

Serum creatinine is the end product from the metabolic break down of creatine phosphate. It is removed from the blood chiefly by the kidneys,

Table 1: Effect of EBBM on change in bwt, Kidney weight and urine volume against cisplatin induced nephrotoxicity

Group	Change in body weight (g)	Change in kidney weight (g)	Urine volume (ml)
I-Positive Control	0.430±0.017***	0.430±0.017***	10.90±0.433***
II-Negative control	0.753±0.021	0.753±0.021	5.58±0.560
III-EBBM 300 mg/Kg bwt	0.450±0.017***	0.450±0.017***	9.26±0.286***
IV-Prophylactic EBBM 150 mg/Kg bwt	0.545±0.014***	0.545±0.014***	8.53±0.255***
V-Prophylactic EBBM 300 mg/Kg bwt	0.483±0.015***	0.483±0.015***	9.46±0.157***
VI-Curative EBBM 150 mg/Kg bwt	0.704±0.020**	0.704±0.019	7.43±0.380***
VII-Curative EBBM 300 mg/Kg bwt	0.619±0.021***	0.619±0.021***	7.78±0.381***

EBBM: Ethanolic extract of the bark of *Bassia malabarica*, SEM: Standard error of mean, bwt: Body weight. ***Change in activities at $p<0.001$ when compared to negative control group, **$p<0.01$, *$p<0.05$. Values are expressed as mean±SEM (n=8)

Table 2: Effect of EBBM on serum parameters against cisplatin induced nephrotoxicity

Group	Serum total protein (g/dl)	Creatinine (mg/dl)	Urea level (mg %)	Uric acid level (mg/dl)	BUN (mg %)
I-Positive control	9.73±0.310***	0.68±0.097***	39.6±2.060***	1.70±0.115***	18.5±0.961***
II-Negative control	3.51±0.391	2.91±0.142	73.5±2.720	3.95±0.146	33.0±1.580
III-EBBM 300 mg/Kg bwt	9.14±0.174***	0.93±0.144***	41.7±2.090**	1.80±0.160***	19.5±0.976***
IV-Prophylactic EBBM 150 mg/Kg bwt	6.73±0.129***	1.27±0.068***	40.5±5.260*	1.85±0.165***	21.7±0.863***
V-Prophylactic EBBM 300 mg/Kg bwt	8.01±0.34***	1.07±0.130***	42.3±2.060***	1.66±0.121***	19.8±0.964***
VI-Curative EBBM 150 mg/Kg bwt	4.273±0.333	2.63±0.173	66.9±2.790	3.88±0.145*	31.7±1.430
VII-Curative EBBM 300 mg/Kg bwt	5.74±0.369***	2.02±0.186***	58.5±3.690**	2.33±0.162***	27.4±1.720*

EBBM: Ethanolic extract of the bark of *Bassia malabarica*, SEM: Standard error of mean, bwt: Body weight, BUN: Blood urea nitrogen. ***Change in activities at p<0.001when compared to negative control group, **p<0.01, *p<0.05. Values are expressed as mean±SEM (n=8)

Table 3: Effect of EBBM on *in vivo* antioxidants against cisplatin induced nephrotoxicity

Group	LPO (n mol/mg of tissue)	GSH (n mol/mg of tissue)	CAT (n mol/mg of tissue)
I-Positive control	0.26±0.006***	0.70±0.004***	0.84±0.003***
II-Negative control	0.94±0.005	0.35±0.004	0.47±0.004
III-EBBM 300 mg/Kg bwt	0.34±0.006***	0.65±0.004***	0.79±0.004***
IV-Prophylactic EBBM 150 mg/Kg bwt	0.55±0.006***	0.53±0.003	0.64±0.003***
V-Prophylactic EBBM 300 mg/Kg bwt	0.47±0.005***	0.60±0.003***	0.68±0.005***
VI-Curative EBBM 150 mg/Kg bwt	0.82±0.007***	0.42±0.004***	0.52±0.004***
VII-Curative EBBM 300 mg/Kg bwt	0.66±0.008***	0.47±0.004***	0.58±0.005***

EBBM: Ethanolic extract of the bark of *Bassia malabarica*, SEM: Standard error of mean, bwt: Body weight, GSH: Glutathione, LPO: Lipid peroxidation, CAT: Catalase. ***Change in activities at p<0.001 when compared to negative control group, **p<0.01, *p<0.05. Values are expressed as mean±SEM (n=8)

primarily by glomerular filtration. Serum urea is an important indicator of renal diseases and is an end product of protein metabolism. It is the nitrogenous waste product that is excreted by the kidney and serum uric acid is the end product of purine catabolism. In disease condition glomerular filtration is decreased thus elevating the levels of serum creatinine, serum urea and serum uric acid levels. In the agreement with our results, Mansour *et al*. [21] and Sueishi *et al.* [24] reported that the nephrotoxic effect of cisplatin was evidenced by a significant increase of creatinine and BUN levels. The increased creatinine level in cisplatin treated rats might be due to glomerular damage as a result of ROS generation [21,24].

Effect of EBBM on in vivo antioxidants against cisplatin induced nephrotoxicity

Cisplatin decreases antioxidants and antioxidant enzymes leading to enhanced generation of ROM and LPO. Cisplatin inhibits the activity of antioxidant enzymes in renal tissue like reduced GSH and CAT. Treatment groups may inhibit LPO by scavenging free radicals and increasing the intracellular concentration of reduced GSH and CAT. Improvement of renal GSH in EBBM extract treated groups was observed in prophylactic EBBM 300 mg/Kg bwt (Group V) when compared to the negative control (Group II) as shown in Table 3.

The ROS inhibits the activity of the antioxidant enzymes in renal tissues with increased LPO and nephrotoxicity [25]. This oxidative stress inhibits the antioxidant enzymes and generates the ROS that destroys the lipid, protein and DNA components of the cell with subsequent enzymatic inactivation and mitochondrial dysfunction. In the present study, the morphological findings of negative control group rats treated with cisplatin revealed glomerular damage. These morphological findings could explain the disturbances of both glomerular and tubular functions, where these disturbances might be the main cause of the reduction of bwt, increase the relative kidney weight, elevation of creatinine and BUN levels [26].

CONCLUSION

Results revealed a marked protection in the prophylactic group by the administration of EBBM against cisplatin induced necrotic damage in nephrons. The effect of EBBM was evident by the decrease of serum creatinine, urea, uric acid and BUN indicating the increased glomerular filtration rate.

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