

Effect of Hydroalcoholic Extract of Boerhaavia Diffusa Linn against Cisplatin Induced Nephrotoxicity

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Abstract

Objective: To evaluate the Nephroprotective effect of Hydroalcoholic extract of Boerhaavia diffusa (HAEBD) in Cisplatin induced acute failure in rats.

Materials & Methods: Adult female Wistar rats were divided into five groups. G1(Normal Control), G2(Toxic control), G3(Perse control with silymarin), HAEBD (200mg/kg and 400mg/kg) , were administered orally to G4 & G5. Cisplatin was used to induce acute renal failure. The parameters studied included Serum creatinine, BUN, Urea, alkaline phosphatase & markers of oxidative stress such as renal malondialdehyde (MDA), superoxidase dismutase(SOD), glutathione(GSH), Glutathione peroxidase(GPx), catalase(CAT) in renal cortical homogenates. Histopathological examination also carried out

Results: The results revealed that HAEBD treatment significantly reduced blood urea and serum creatinine levels elevated by CP administration. Furthermore HAEBD significantly attenuated CP induced increase in MDA & decrease in reduced GSH, and CAT & SOD and GSH peroxidase activities in renal cortical homogenates. Additionally histopathological examination showed that HAEBD markedly ameliorated CP induced renal tubular necrosis.

Conclusion: The results indicate that the aerial parts of Boerhaavia diffusa are endowed with Nephroprotective effect.

Key words: Cisplatin, Boerhaavia diffusa, lipid peroxidation, Nephrotoxicity.

I. Introduction

Cisplatin (cis-diammine dichloroplatinum II (CDDP)) is a chemotherapeutic agent that is used for the treatment of a wide variety of cancers.¹⁻² One of the limiting side effects of cisplatin use is nephrotoxicity and high dose of CDDP produce the impairment of kidney, causes decrease in renal blood flow, glomerular filtration rate and increases urea and creatinine level in blood.³ Various studies have revealed that cisplatin induced renal damage is due to the involvement of oxidative stress via free radical formation which in turn produces impairment in proximal tubular reabsorption of water, sodium ions (Na⁺) and glucose⁴. The cisplatin induced nephrotoxicity was characterized by signs of injury such as changes in urine volume, body weight, increase the products of lipid peroxidation, and change renal clearance.⁵ Acute kidney injury remains a significant cause of increased morbidity and mortality among patients, particularly in critical care units. Many herbal remedies have been employed in various medical systems for the treatment and management of Nephrotic disorder. Boerhaavia Diffusa belongs to the family Nyctaginaceae, which is commonly known as Horse-purslane, Hogweed and Pig weed in English indigenous to India, exhibits a wide range of medicinal properties as per Ayurvedic claims. Different parts of the B. diffusa have been widely used for the treatment of dyspepsia, jaundice, enlargement of spleen, abdominal pain, abdominal tumors, and urinary disorders used in the traditional medicine.⁶ Pharmacological studies have demonstrated that B.diffusa known to possess diuretic⁷; nephrotic syndrome⁸; anti-inflammatory and anti-nociceptive⁹; anticonvulsant¹⁰; immunomodulatory¹¹; hepatoprotective¹²; antiurolithiatic¹³; antioxidant and antidiabetic activity.¹⁴ Due to the combination of diuretic, antioxidants and anti-inflammatory activities, B. diffusa regarded as therapeutically highly efficacious for the treatment of inflammatory renal diseases and common clinical problems such as nephrotic syndrome, oedema, and ascites. The whole plant analysis of B. diffusa is known to contain numerous phytochemicals constituents that include flavonoids, alkaloids, triterpenoids, steroids, lipids, lignins, tannins, phlobaphenes and ursolic acid¹⁵⁻¹⁷. From these investigations it is believed that B. diffusa improves renal function and may protect renal cell against chemical induced nephrotoxicity. Therefore, the present investigation is to assess the traditional use of Boerhaavia diffusa Linn. in treating cisplatin induced Nephrotoxicity.

II. Experimental Methods

Materials & Methods

Whole plant of Boerhaavia diffusa were collected from local traders, Tamilnadu, shed dried for a week in a shadow and blended to coarse powder. About 500gm of dried fine powder of Boerhaavia diffusa were

soaked in the extractor and macerated for 30 hrs with petroleum ether. There it is reflexed successfully with chloroform, after that it is extracted with alcohol and water by continuous hot percolation method using soxhlet apparatus for 40hrs separately. Hydro alcoholic extracted was filtered and concentrated in vacuum using rotary flask evaporator under reduced pressure. After concentration hydro alcoholic extract of Boerhaavia diffusa given brownish residue stored in air tight container.

Animals

In-house laboratory bred 6 week old wistar rats were selected for the study. Animals were maintained under controlled temperature at $20 \pm 2^{\circ}\text{C}$ and relative humidity of 50-60% with an alternating 12hr light/ dark cycle. The animals were acclimatized for 1 week before the study and had free access to standard laboratory feed and water ad libitum. The research work was approved by Institutional animal Ethical Committee.(IAEC/KMCP/61)

Experimental Protocol

Kidney injury was induced by a single intraperitoneal (i.p.) injection of cisplatin (Sigma Chemical Co, USA) (5 mg/kg b.w.) [30]. Rats were divided into 5 groups of 6 each. **Group 1:** Served as Normal Control, which received 10ml/kg of normal saline. **Group 2:** Served as Toxic control, which received Cisplatin (5mg/kg b.w, i.p) on day one. **Group 3:** Served as positive control which received Cisplatin (5mg/kg b.w, i.p) on day one followed by silymarin (50mg/kg b.w, orally) for 10 days. **Group 4:** Served as Treatment group, which received Cisplatin (5mg/kg b.w,i.p) single dose on the day one followed by HAEBD at a dose of 200mg/kg orally for 10 days. **Group 5:** Served as Treatment group, which received Cisplatin (5mg/kg b.w,i.p) single dose on the day one followed by HAEBD at a dose of 400mg/kg orally for 10 days.

Biochemical Assay

On day 11 cisplatin injection was administered to all groups except normal control. After 72 hrs of cisplatin injection animals were sacrificed using ether anesthesia;). Blood samples were withdrawn from retro-orbital plexus under light ether anesthesia without any anti-coagulant and allowed for 10 minutes to clot at room temperature. It was centrifuged at 2500 rpm for 20 minutes for serum separation. The serum obtained was kept at 4°C until used. Studies have been carried out to determine kidney function markers such as urea, BUN, creatinine, albumin, calcium and magnesium and uric acid and serum lipid levels like total cholesterol, LDL-cholesterol and triglycerides were estimated from serum sample using standard diagnostic kit (SPAN Diagnostics and Crest Biosystems, India).

Estimation of Biomarkers of Oxidative Stress

Blood samples were taken for serum analyses and the kidneys were removed for histological studies. Kidneys washed with ice –cold normal saline and homogenates (10% w/v) were prepared in PBS. A part of the homogenate was used for the estimation of glutathione (GSH) and lipid per oxidation. The remaining homogenate was centrifuged at 5000 rpm for 10 min at 4°C ; after removal of the cell debris, the supernatant was used for the assay of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX).

Serum creatinine was assayed according to Jaffe's kinetic method,^[20] Blood urea nitrogen and urea was assayed according to Berthelot end point assay^[21] and Alkaline phosphatase according to pNPP- AMP (IFCC), kinetic assay^[22] using Autospan kits.

The GSH level was measured colorimetrically using 5, 5'- Dithio.bis (2 - nitrobenzoic acid) (DTNB) as the substrate. The concentrations of malondialdehyde (MDA) as indices of lipid peroxidation were assessed. The SOD activity was determined by the Nitro blue tetrazolium (NBT) reduction method. The GPx activity was determined by the method. The CAT activity was determined from the rate of decomposition of H_2O_2 , method

Histopathological Studies

For light microscopic evaluation, kidney tissues of each group were fixed in 10% phosphate buffered formalin. Paraffin-embedded specimens were cut into 6 mm-thick sections and the kidney sections were stained with hematoxylin and eosin and were observed under light microscope for any histopathological changes. . Histopathological studies were done at Apollo diagnostics, Madurai.

Statistical Analysis

The results were expressed as Mean \pm S.E.M and analyzed with one way analysis of variance between the two groups and followed by Newman-Keuls' multiple range tests. Probability values $p \leq 0.05$ were considered significant.

Table 1

Effect of HAEBD on serum creatinine, blood urea nitrogen & alkaline phosphatase:

| Group No. | TREATMENT DOSE (mg/Kg) | Serum creatinine | Blood urea nitrogen mg/dl | Alkaline phosphate mg/dl | Urea mg/dl |
|-----------|--|--------------------------|---------------------------|---------------------------|-------------------------|
| I | Normal control 10ml/kg normal saline | 0.05±0.02 | 18.55±0.58 | 155.60±0.75 | 17.2±0.40 |
| II | Toxic control 7.5mg/kg Cisplatin induced | 1.310±0.05 ^{*a} | 104.30±5.55 ^{*a} | 345.20±1.58 ^{*a} | 78.6±1.65 ^{*a} |
| III | Positive control 50mg/kg Silymarin | 0.796±0.03 ^{*b} | 38.75±1.28 ^{*b} | 186.75±0.90 ^{*b} | 24.5±0.48 ^{*b} |
| IV | Treatment control HAEBD 200 mg/kg | 0.870±0.04 ^{*b} | 56.10±2.05 ^{*b} | 248.60±1.05 ^{*b} | 46.4±0.56 ^{*b} |
| V | Treatment control HAEBD 400mg/kg | 0.805±0.03 ^{*b} | 44.25±1.60 ^{*b} | 210.45±0.96 ^{*b} | 8.2±0.50 ^{*b} |

- Values are expressed as Mean ± SEM.
- Values are found out by using one way ANOVA followed by Newmannkeul's multiple range tests.
- *a – values are significantly different from Normal control at P < 0.01.
- *b – values are significantly different from Toxic control(G2) at p < 0.01.

Table 2Effects of Hydroalcoholic extract of Boerhaavia diffusa on renal malondialdehyde, glutathione, catalase, and superoxide dismutase, glutathione peroxidase (gp_x) activities in treated rats.

| Group No. | TREATMENT DOSE (mg/Kg) | MDAu/mg Protein | GSH u/mg Protein | CAT u/mg protein | SODu/mg Protein | GPX u/mg Protein |
|-----------|--|-------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| I | Normal control 10ml/kg normal saline | 2.11±0.18 | 15.15±0.40 | 55.30±1.40 | 15.30±0.40 | 23.20±0.90 |
| II | Toxic control 7.5mg/kg Cisplatin induced | 4.90±0.42 ^{*a} | 4.40±0.20 ^{*a} | 10.45±0.35 ^{*a} | 6.15±0.16 ^{*a} | 10.10±0.32 ^{*a} |
| III | Positive control 50mg/kg Silymarin | 2.85±0.24 ^{*b} | 13.40±0.36 ^{*b} | 40.90±0.75 ^{*b} | 13.05±0.32 ^{*b} | 20.15±0.56 ^{*b} |
| IV | Treatment control HAEBD 200 mg/kg | 3.65±0.32 ^{*b} | 10.52±0.26 ^{*b} | 29.45±0.45 ^{*b} | 10.90±0.25 ^{*b} | 16.40±0.62 ^{*b} |
| V | Treatment control HAEBD 400mg/kg | 3.05±0.26 ^{*b} | 11.25±0.30 ^{*b} | 34.50±0.68 ^{*b} | 12.60±0.28 ^{*b} | 18.25±0.68 ^{*b} |

- Values are expressed as Mean ± SEM.
- Values are found out by using one way ANOVA followed by Newmannkeul's multiple range tests.
- *a – values are significantly different from Normal control at P < 0.01.
- *b – values are significantly different from Toxic control(G2) at p < 0.01

In the present investigation, administration of single injection of cisplatin (7mg/kg) cause a marked reduction in renal function, shows significant rise in BUN, Creatinine & uric acid as compared to control group. The pre-treatment with Hydro alcoholic extract of Boerhaavia diffusa (HAEBD) p.o. significantly (P < 0.01) lowered the elevated serum urea creatinine, and Blood urea nitrogen (BUN) when compared to the cisplatin group. (Table:1) The pre-treatment with silymarin showed a marked decrease in concentrations of blood urea, serum creatinine as compared to control group.

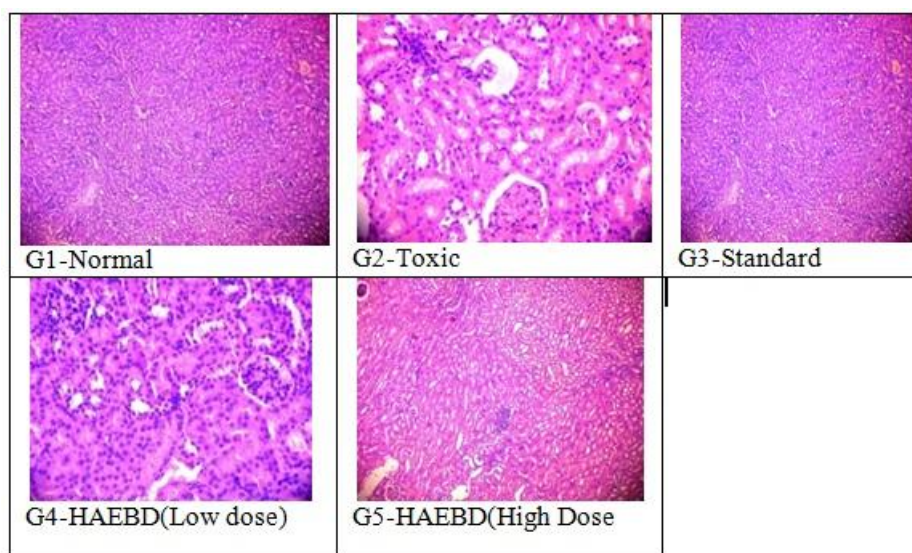
Effects of HAEBD on renal oxidant/antioxidant status

In cisplatin treated group the activities of antioxidant enzymes like SOD, CAT and GPx and levels of GSH were found to be significantly decreased with marked increase in MDA as compared with control (P < 0.01). The pre-treatment of HAEBD and silymarin was found to significantly elevate the decreased activities of SOD, CAT and GPx (P < 0.01). The activities of renal SOD, CAT and GPx in the cisplatin treated, cisplatin plus

HAEBD and cisplatin plus silymarin administered groups were given in [Table no:2]. Administration of HAEBD and silymarin also inhibited the cisplatin-induced elevation in the MDA. The decrease in the GSH levels in renal tissues induced by cisplatin was prevented by the administration of HAEBD and silymarin ($P < 0.01$) [Table no:2]

Effects of HAEBD on kidney histology

Treatment with cisplatin caused a marked necrosis in proximal tubules and degeneration of the tubular epithelial cells [Figure no:] The pre-treatment with HAEBD and silymarin decreased the cisplatin induced tubular necrosis when compared with cisplatin treated group. G1- Section of normal rat kidney showing normal organization of tubular epithelial cells and glomeruli. G2- Section of rat kidney treated with cisplatin showing acute tubular necrosis with marked congestion and atrophy of glomerulus and infiltration of tubular cells. G3- Section of rat kidney treated with standard showing normality of tubular epithelial cells and glomeruli. G4- Section of rat kidney treated with **HAEBD** extract (low dose) shows mild degenerative changes in tubular epithelial cells. G5- Section of rat kidney treated with **HAEBD** extract (high dose) showing regenerative changes in glomerulus and tubular.



III. Discussion

The impairment of kidney function by cisplatin is recognized as the main side effect and the most important dose limiting factor associated with its clinical use. Several investigators^[23,24] reported that the alterations induced by cisplatin in the kidney functions were characterized by signs of injury, such as increase of products of lipid peroxidation (LPO) and changes in GSH levels in kidney tissue, creatinine and urea levels in plasma.

The renal antioxidant status, such as SOD, CAT, GPx activities, and reduced GSH concentration are significantly decreased in the cisplatin treated group of animals compared to the control group. The declined antioxidant status partially explains the mechanism of nephrotoxicity induced by cisplatin. The renal accumulation of platinum and covalent binding of renal protein may also play a role in the nephrotoxicity.^[25, 26] In the present study, increased serum creatinine and urea were observed in cisplatin treated rats may be due to reduction in glomerular filtration rate. The impairment in kidney function was accompanied by an increase in MDA concentrations in kidney tissue. The above findings were well-correlated with the renal histological results. These observations indicated that cisplatin induced nephrotoxicity and the results are in accordance with previous findings. The pre-treatment of HAEBD provides a significant protection against cisplatin-induced nephrotoxicity with lowering the level of plasma creatinine and blood urea in cisplatin treated animals.

Decreased concentration of GSH increases the sensitivity of organs to oxidative and chemical injury. The role of GSH, non-protein thiols in the cells, in the formation of conjugates with electrophilic drug metabolites, most often formed by cytochrome P-450-linked monooxygenase, is well-established.^[27] Studies with a number of models show that the metabolism of xenobiotics often produced GSH depletion. Reduced renal GSH can markedly increase the toxicity of cisplatin. The depletion of GSH also seems to be a prime factor that permits lipid peroxidation in the cisplatin-treated group. Moreover, the protection of GSH is also by forming the substrate for the GPx activity that can react directly with various aldehydes produced from the

peroxidation of membrane lipids. The enhanced GPx activity could partially explain the protection of biomembranes from oxidative attack.

Decreased SOD activity could cause the initiation and propagation of lipid peroxidation in the cisplatin treated group. This may be either due to loss of copper and zinc, essential for the activity of enzyme or due to ROS induced inactivation of enzyme proteins. The decrease in activities of CAT and GPx could enhance the lipid peroxidation. Thus, the levels of MDA, as a result of lipid peroxidation, were increased in the cisplatin-treated animals. Although, the exact mechanism of cisplatin-induced nephrotoxicity is not well-understood, several investigators have shown that cisplatin nephrotoxicity is associated with LPO in renal tissue. LPO is ascribed to a free radical-mediated chain reaction that damages cell membranes, and inhibition of this process by HAEBD is mainly attributed to the ability of scavenger free radicals.^[28] In the present investigation, pretreatment with HAEBD inhibited the increase in LPO induced by cisplatin in renal tissue, indicating antioxidant activity of HAEBD.^[29]

The histopathological evaluation of the kidney preparations in treatment group also revealed a decreased cisplatin-induced tubular necrosis. Cisplatin-induced renal damage is associated with increased renal vascular resistance and histopathological damage to proximal tubular cells. On the other hand, an increase in GSH levels in the renal tissue indicates that pretreatment with HAEBD was due to oxidative stress. The effects of HAEBD on cellular GSH may be due to antioxidant effects. The treatment with HAEBD prevented the lipid peroxidation by enhancing the renal CAT, SOD and GPx activities.

IV. Conclusion

In conclusion, it was shown that cisplatin treatment induced renal damage and pretreatment with hydro alcoholic extract of Boerhaavia diffusa (HAEBD) provided protective effect against this cisplatin-induced nephrotoxicity. However, before concluding a potential usefulness of hydro alcoholic extract of Boerhaavia diffusa(HAEBD) as adjunct to the cisplatintherapy, further clinical investigation is needed.

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