

Original Article

NEPHROPROTECTIVE ACTIVITY OF PLUMERIA RUBRA AGAINST CISPLATIN INDUCED NEPHROTOXICITY IN EXPERIMENTAL RATS

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ABSTRACT

Objective: The current study was designed to evaluate the protective effect of standardized hydroalcoholic extract of *Plumeria rubra* (HAEPR) against cisplatin-induced nephrotoxicity in Wistar rats.

Methods: HAEPR was administered orally at 3 dose levels (100,200,400 mg/kg). Vitamin E (250 mg/kg) was used as a Standard nephroprotective agent. The kidney function test (estimation of serum creatinine, albumin, blood urea nitrogen) oxidative stress study (estimation of superoxide dismutase, malondialdehyde activity) and histological examination of kidneys was conducted.

Results: The efficacy of HAEPR was compared with Cisplatin (CP) treated group. Serum creatinine and BUN was significantly ($p < 0.01$) elevated in CP-treated group compared to the control group. HAEPR (100,200 mg/kg) and Vitamin E (250 mg/kg) significantly ($p < 0.01$) decreased the serum creatinine and BUN levels. CP treated group exhibited significant ($p < 0.01$) decrease in albumin when compared to control. Significant ($p < 0.01$) increase in the serum albumin level was found in HAEPR (100,200 mg/kg) and Vitamin E (250 mg/kg) compared to CP group. Significant ($p < 0.01$) decrease in the activity of SOD was observed in the CP group as compared to control. HAEPR (100 and 200 mg/kg) and Vitamin E (250 mg/kg) significantly ($p < 0.01$) increased SOD levels. HAEPR (400 mg/kg) significantly ($p < 0.05$) increased SOD levels. HAEPR (100,200,400 mg/kg) significantly ($p < 0.01$) decreased MDA levels as compared to CP group. Histopathological examination of the kidneys showed that HAEPR markedly ameliorated Cisplatin-induced renal tubular necrosis. An extract was found effective at all doses, although low dose (100 mg/kg) was found to be more effective and comparable with the standard group (Vitamin E 250 mg/kg).

Conclusion: Present investigation revealed that HAEPR resulted in attenuation of Cisplatin-induced renal damage in rats.

Keywords: *Plumeria rubra*, Cisplatin, Nephrotoxicity, Creatinine, Oxidative stress

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INTRODUCTION

The urinary tract is the body's drainage system of excretion of urine that is composed of wastes and extra fluid. It causes the elimination of nitrogenous waste produced by protein digestion from the bloodstream. The urinary system possesses the ability to distinguish between useful and toxic compounds in the blood that should be maintained or eliminated. Kidneys play an important role in maintaining normal blood pH by eliminating and maintaining acidic and basic compounds in the blood. The functional units called nephrons are capable of distinguishing between the different compounds dissolved in the blood, and eliminating only those that are not beneficial [1]. Numerous conditions like Diabetes, hypertension, Glomerulonephritis autoimmune disease result in damage to the kidneys, thus affecting their ability to filter waste from the blood [2]. Cisplatin exerts dose dependant nephrotoxicity which limits its clinical usage in cancer chemotherapy. It induces apoptosis and necrosis of renal tubular cells by activation of extrinsic and intrinsic mitochondrial pathways. It also involves p53 mediated proapoptotic activation of proinflammatory pathways. Proinflammatory pathways activation and infiltration of inflammatory cells are major mechanisms in cisplatin-induced nephrotoxicity [3]. The kidney accumulates cisplatin to a greater extent unlike other organs and is the major route for its excretion. The cisplatin concentration in proximal tubular epithelial cells is about 5 times the serum concentration [4]. The disproportionate accumulation of cisplatin in kidney tissue contributes to cisplatin-induced nephrotoxicity [5]. Cisplatin is a strong cellular toxin and nephrotoxicity is one of the most important complications of this drug in clinical and experimental models. The highest concentration of cisplatin is observed in mitochondria, nuclei, cytosol and microsomes. Nephrotoxicity induced by CP is mediated by mitogen-activated protein kinase (MAPK) intracellular signaling pathways. The MAPK pathways are activated by diverse extracellular

physical and chemical stresses that regulate cell proliferation, differentiation, and survival [6]. Primary targets of cisplatin in the kidney are the proximal and distal convoluted tubules where it accumulates and promotes cellular damage, by multiple mechanisms including oxidative stress, DNA damage and apoptosis [6]. Cisplatin is interlaced to glutathione and metabolized through a gamma-glutamyl transpeptidase and cysteine S-conjugate β -lyase-dependent pathways to a reactive thiol. The kidney accumulates cisplatin by peritubular uptake and concentration of the drug in the renal cortex is several folds greater than other organs [7]. Platinum compounds mediate their cytotoxic effects by interaction with DNA. In an aqueous environment, the chloride ligands of cisplatin are replaced by water molecules generating a positively charged electrophile. The electrophile reacts with nucleophilic sites on intracellular macromolecules to form DNA, RNA, and protein adducts. Cisplatin binds to DNA leading to the formation of inter and intrastrand cross-links, thereby arresting DNA synthesis and replication in rapidly proliferating cells [8, 9].

A large number of medicinal plants are claimed to be useful in renal failure in all traditional systems of medicine and folklore. *Plumeria rubra* trees are found throughout in India and in tropical areas. Though the plant and its extracts have been extensively used in the folklore medicines, information from an organized search of published literature does not provide the evidence for its nephroprotective activities. In the present study, we have investigated the nephroprotective activity of hydroalcoholic extract of the flowers of *Plumeria rubra* in Wistar rats. *Plumeria* species have been reported to have antimicrobial [10, 11] anticancer [12-14] antidiabetic activities [15, 16]. It has been identified as having potential antioxidant property. The antioxidant capacities of plant extracts are due to their radical scavenging activity, binding of transition metal ion catalysts, increasing endogenous status of antioxidant enzymes to prevent oxidative damage [17].

MATERIALS AND METHODS

Chemicals

Standardized hydroalcoholic extract of *Plumeria rubra* (HAEPR) was procured from Shamantak enterprises, Pune. Cisplatin cis-diamineplatinum (II) dichloride was purchased from S. K. Enterprises, Pune. All other chemicals used were of the analytical grade.

Animals

Thirty-six Wistar male and female albino rats, weighing 200-250g, were used for the study. The animals were maintained under standard laboratory conditions with controlled temperature (20 ± 2 °C) and humidity (60%) with regular light cycle (12 light/12 dark). The animals were acclimatized for 1 w before the study and had free access to standard laboratory food and water ad libitum. All experimental procedures were conducted in accordance with the principles for the care and use of laboratory animals in research and approved by the institutional animal ethics committee. (ACP/IAEC/2018/01).

Experimental design

The nephroprotective activity was tested on six groups of albino Wistar rats (3 males+3 females) each group consisting of six animals.

Group I-Served as control received normal saline (0.9%; p. o)

Group II-Toxic control rats received normal saline (0.9%; p. o) and cisplatin (6 mg/kg; i. p)

Group III-Received Vitamin E 250 mg/kg as Standard nephroprotective agent and cisplatin (6 mg/kg; i. p)

Group IV-Received HAEPR (100 mg/kg; p. o) and cisplatin (6 mg/kg; i. p)

Group V-Received HAEPR (200 mg/kg; p. o) and cisplatin (6 mg/kg; i. p)

Group VI-Received HAEPR (400 mg/kg; p. o) and cisplatin (6 mg/kg; i. p)

On the 15th day, 2 h after the administration of extracts and normal saline groups II-VI received CP (6 mg/kg; i. p). At the end of the experimental period, i. e on the 16th-day rats were sacrificed by cervical dislocation. The blood was collected in an anticoagulant tube and allowed to stand for 30 min at 37 °C and then centrifuged to separate the serum to evaluate the biochemical markers [18-20]. Serum MDA was measured using the method of Buege. In this method, 100 μ L serum was diluted to 500 μ L distilled water. The samples were kept in a boiling water bath for 15 min. To the diluted sample 1 ml of Trichloroacetic acid TCA-2-thiobarbituric acid (TBA) HCl reagent was added. The reaction mixture was cooled and centrifuged. The supernatant was taken and the optical density of the pink color formed was read at 535 nm. The optical density of the pink color formed was directly proportional to the concentration of serum MDA in the given sample [21]. Serum superoxide dismutase activity was estimated by Marklund and Marklund method. In this method superoxide anion is involved in auto-oxidation of pyrogallol at alkaline pH (8.5). The superoxide dismutase (SOD) inhibits auto-oxidation of pyrogallol. This can be determined as an increase in absorbance at 420 nm. [22]. Jaffe's Alkaline picrate method was used for creatinine estimation. Creatinine reacts with alkaline picrate to give

orange color. Intensity of the color formed was directly proportional to the amount of creatinine present in the sample. Albumin determination was done by the Biuret method. Albumin present in globulin, free solution react with copper sulphate in an alkaline medium to give violet color [23]. The blood urea nitrogen was estimated by the Berthelot method. Urease catalyses the conversion of urea to ammonia and carbon dioxide. The ammonia, thus released reacts with a mixture of salicylate, hypochlorite and nitroprusside to yield indophenol, a blue-green colored compound. The intensity of the color produced is directly proportional to the concentration of urea in the sample and was measured spectrophotometrically at 578 nm [24].

Histopathology

Fixation of the kidney was done by cutting and fixing in Bouin's fluid immediately after removal from the animal body. The tissues were fixed in Bouin's fluid for about 24 h. The tissues were then taken and washed in glass distilled water for a day to remove excess of picric acid. This was followed by Dehydration in which the tissues were kept in the following solutions for an hour each; 30%, 50%, 70% and 100% alcohol. Xylene was used as the clearing agent, for one or two hours, two or three times. The tissues were taken out of xylene and were kept in molten paraffin embedding bath with molten wax maintained at about 50 °C. A clear glass plate was smeared with glycerine. L-shaped mould was placed on it to form a rectangular cavity. The molten paraffin wax was poured and air bubbles were removed by using a hot needle. The tissue was placed in the paraffin and oriented with the surface to be sectioned. Then the tissue was pressed gently towards the glass plate to make settle uniformly with a metal pressing rod and allowed the wax to settle and solidify room temperature. The paraffin block was kept in cold water for cooling. Section cutting was done with a rotatory microtome. The excess of paraffin around the tissue was removed by trimming, leaving $\frac{1}{2}$ cm around the tissue. Then the block was attached to the gently heated holder. To produce uniform sections, the microtome knife was adjusted to the proper angle in the knife holder with only the cutting edge coming in contact with the paraffin block. The tissue was cut in the thickness range of about 7 μ m. The sections were spread on a warm water bath after they were detached from the knife with the help of hair brush. Required sections were spread on a clean slide and kept at room temperature. The sections were stained as follows; deparaffinization with xylene two times each for five minutes. Dehydration through descending grades of ethyl alcohol. Staining with Ehrlich's Haematoxylin was done for 15-20 min. Then the sectioned tissues were thoroughly washed in tap water for 10 min. Rinsed with distilled water and stained with Eosin. Dehydration again with ascending grades of alcohol. Finally, the tissues were cleared with xylene two times, each for about 3 min interval. On the stained slide, DPX mountant was applied uniformly and micro glass cover slides were spread. The slides were observed in Nikon microscope and microphotographs were taken [25].

Statistical analysis

The results were expressed as mean \pm SEM. Comparison between the groups was made by one-way analysis of variance (ANOVA) followed by "Dunnett's Test". P<0.05 was considered to be significant.

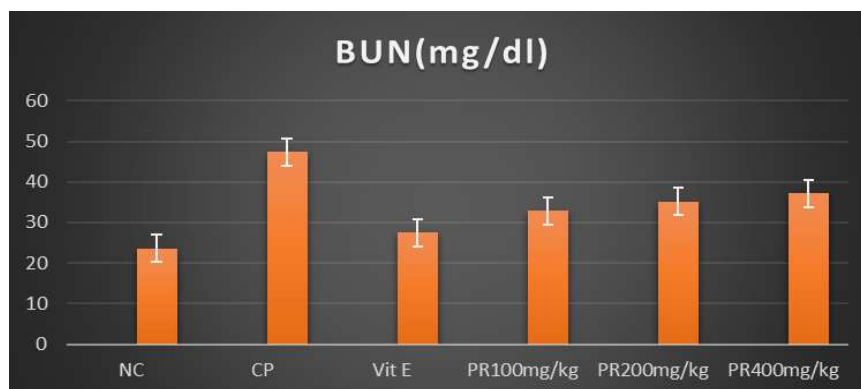


Fig. 1: Effect of HAEPR on BUN

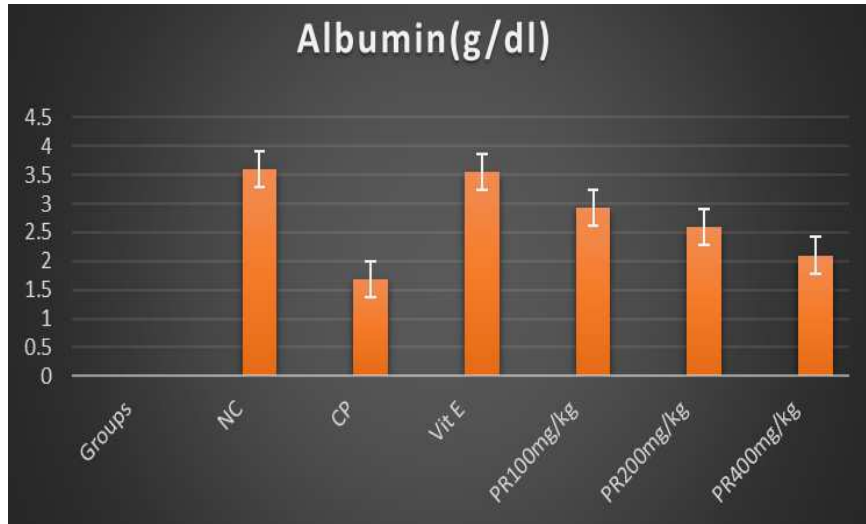


Fig. 2: Effect of HAEPR on Albumin

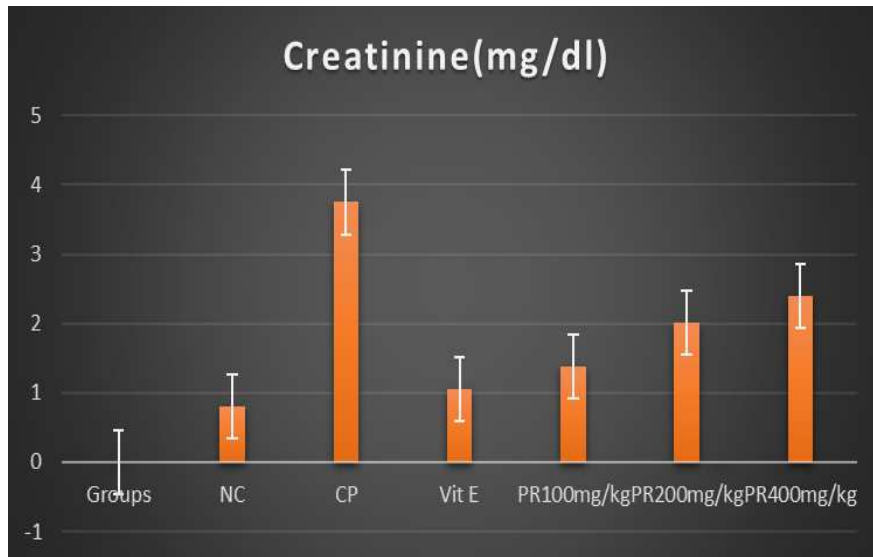


Fig. 3: Effect of HAEPR on creatinine

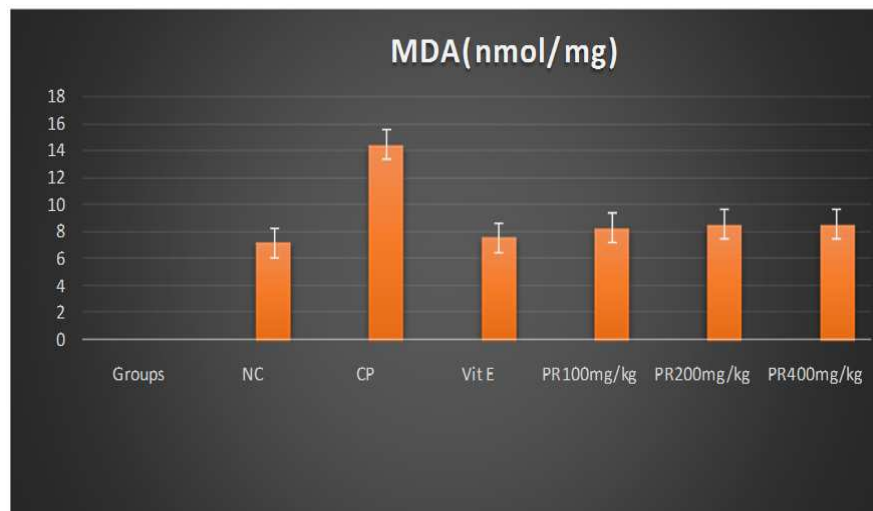


Fig. 4: Effect of HAEPR on MDA

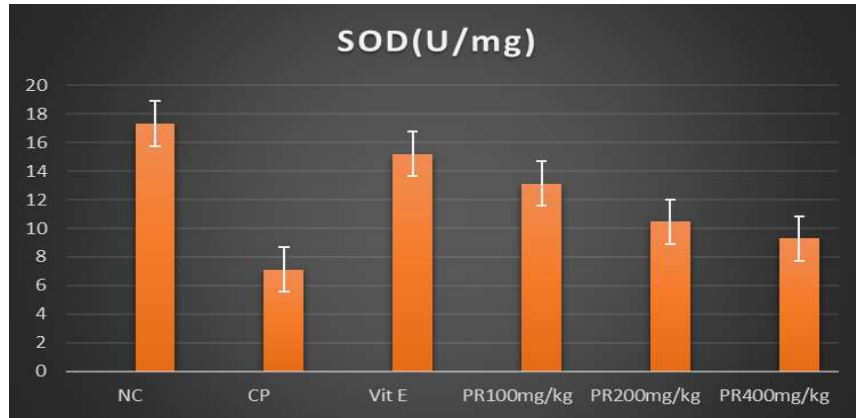


Fig. 4: Effect of HAEPR on SOD

RESULTS

Biochemical analysis

Serum creatinine was significantly ($p < 0.001$) elevated in the CP group when compared to control group. HAEPR treatment (100,200 mg/kg) and Vitamin E (250 mg/kg) significantly ($p < 0.01$) decreased the serum creatinine levels as compared to the CP group. CP group exhibited significant ($p < 0.001$) decrease in albumin when compared to control. Significant ($p < 0.01$) increase in the serum albumin level was found when compared to the CP group by HAEPR (100,200 mg/kg) and Vitamin E (250 mg/kg) treatment. Serum BUN level was significantly ($p < 0.001$) elevated in the CP group compared with

control group. HAEPR (100,200 mg/kg) significantly ($p < 0.01$) decreased the serum BUN level as compared with the CP control group. HAEPR (100 mg/kg) was comparable to that of the standard group. Significant ($p < 0.001$) decrease in the activity of SOD was observed in the CP group as compared to control. HAEPR (100 and 200 mg/kg) significantly ($p < 0.01$) increased SOD level when compared to the CP group. HAEPR (400 mg/kg) significantly ($p < 0.05$) increased SOD levels. Significant ($p < 0.001$) increase in the activity of MDA was observed in the CP group as compared to control. HAEPR (100,200,400 mg/kg) significantly ($p < 0.01$) decreased MDA levels as compared to the CP group. HAEPR (100 mg/kg) exhibited results similar to that of std Vitamin E.

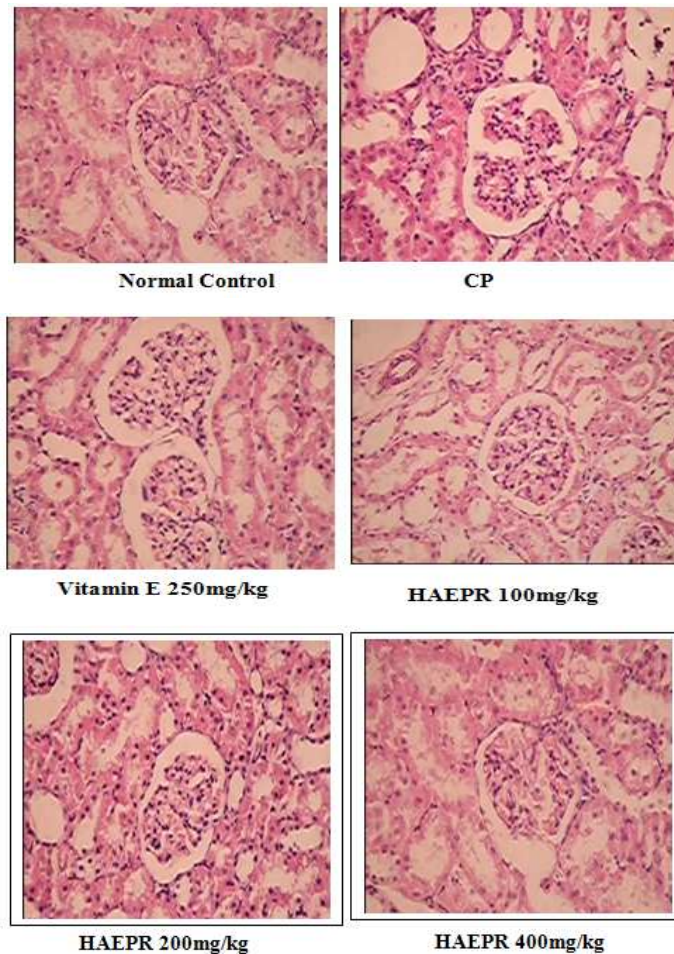


Fig. 6: Effect of HAEAR on histopathology of kidneys

DISCUSSION

Cisplatin is an effective compound in the treatment of several cancers. Its clinical use, however, is associated with severe adverse effects. Main side effect that limits the usage in treatment of cancer is nephrotoxicity. Cisplatin in the kidneys penetrates the tubular cells and reaches high concentration in the proximal tubules. Glomerular injury is less frequent. Tubular damage manifests through impaired reabsorption that is characterized by reduced glomerular filtration rate, increased serum creatinine and blood urea concentrations. The pathophysiological mechanism of cisplatin-induced tubular damage involves a number of interconnected factors like accumulation of cisplatin and conversion into nephrotoxins, DNA damage, mitochondrial dysfunction oxidative stress, inflammatory response, activation of signal transducers and intracellular messengers and activation of apoptotic pathways [26]. The kidney accumulates and retains platinum to a greater degree than other organs and is the principal excretory organ for injected cisplatin. In the rat, the kidney excretes the drug rapidly within the first hour of its administration by a process consisting predominantly of glomerular filtration, with a minor component of secretion. There is no evidence of tubular reabsorption, suggesting that the kidney accumulates cisplatin by peritubular uptake. The uptake of cisplatin by the kidney is dependent on temperature and the normal consumption of oxygen and can be inhibited by drugs that participate in the organic base transport system, suggesting that at least some portion of renal cisplatin uptake is facilitated. Cisplatin predictably lowers glomerular filtration rate in a dose-dependent manner, even after single drug exposure. The onset of renal failure is gradual, usually occurring 3 to 5 d after its administration. Early proteinuria is mild (500 mg/d), as is glycosuria. Enzymuria is common, even in the mildest forms of acute renal failure [5]. The present investigation aims to evaluate the protective effect of HAEPR against cisplatin-induced nephrotoxicity and degree of functional alterations in the kidneys by histopathological and biochemical analysis. Cisplatin (6 mg/kg) was used to induce nephrotoxicity. Vitamin E was one of the antioxidant standard used to ameliorate cisplatin-induced nephrotoxicity in rats. Creatinine is the by-product of muscle metabolism. It is transported from the bloodstream to the kidneys. The kidneys filter most of the creatinine and maintain the normal range of creatinine. Impairment in the kidney function rises creatinine level in the blood causing poor clearance of creatinine. Abnormally high levels of creatinine thus warn of a possible malfunction of the kidneys. Blood urea nitrogen (BUN) is another indicator of kidney function. Urea is also a metabolic byproduct that is elevated if kidney function is impaired [27]. BUN level rises if kidney function decreases. Hypoalbuminaemia is the strongest predictor of death in patients with renal failure. Patients with lower serum albumin level have consistently higher morbidity rates [28]. Reduction in the GFR was indicated by increased levels of creatinine and BUN [29]. A significant decrease in serum albumin also indicated renal impairment. Treatment with the HAEPR at the dose level of 100, 200 and 400 mg/kg body weight significantly lowered the level of creatinine, BUN when compared with the Cisplatin (CP) treated group. It also augmented the reduced levels of albumin. SOD is the primary line of defense against free radical-induced oxidative stress. It causes catalytic dismutation of highly reactive and potentially toxic superoxide radical to hydrogen peroxide [30]. Plants have evolved various protective mechanisms for minimising deleterious effects of free radicals. The enzymatic defence comprises of the efficient antioxidant enzymes such as catalase, peroxidase, superoxide dismutase [31].

Increased ROS production in renal tissue is responsible for damage of organs marked by changes in levels of MDA and SOD. MDA levels were significantly increased in rats treated with CP when compared to control group. HAEPR significantly attenuated the MDA levels in renal tissue probably due to its capacity to scavenge oxygen free radicals in the kidneys. Moreover, it also significantly increased the levels of SOD resulting in improvement of kidney function and histopathology. Impairment in the histological features of the kidneys was substantiated by CP treatment. Our study demonstrated histological changes in proximal and distal convoluted tubules, which were signs of tubular necrosis and atrophy of the vascular component in glomerulus in CP group. Vitamin E treated rat kidney sections showed architecture similar to normal tubules. Recovery of

renal function was observed by treatment of HAEPR evidenced by the regenerative capability of the renal tubules. The experimental results reveal that the nephroprotective activity of the extract is comparable to that of Vitamin E. The activity elicited by the extract might be due to its ability to activate antioxidant enzymes.

CONCLUSION

The study concludes that cisplatin injury evidenced elevated biochemical markers and histopathological features of acute tubular necrosis. The administration of HAEPR resulted in attenuation of CP induced renal damage. The nephroprotective potential may be due to its antioxidant properties. Further studies are required to characterize the phytoconstituents from HAEPR and to study the exact mechanism of action.

AUTHORS CONTRIBUTIONS

Both the authors contributed equally in preparing, editing, and reviewing the article.

CONFLICTS OF INTERESTS

All authors have none to declare

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