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Antioxidant and Nephroprotective Potential of *Aegle marmelos* Leaves Extract

Jaya Dwivedi[a], Manvendra Singh[b], Swapnil Sharma [c], and Sharad Sharma[c]

[a]Department of Chemistry, Banasthali University, Banasthali, Rajasthan, India; [b]Pinnacle Biomedical Research Institute, Bhopal, India; [c]Department of Pharmacy, Banasthali University, Banasthali, Rajasthan, India

ABSTRACT

The nephroprotective activity of hydro-alcoholic (HAEAM) and ethyl acetate (EAEAM) extracts of *Aegle marmelos* leaves were evaluated against nephrotoxicity induced by cisplatin (CP), a widely used chemotherapeutic agent in cancer therapy. Wistar rats were treated with CP (6 mg.kg^{-1}; i.p.). Treatment groups received the same dose of CP, along with HAEAM and EAEAM (200 and 400 mg.kg^{-1}) orally for 5 d. Blood urea nitrogen (BUN), serum creatinine, and antioxidant enzymes were estimated in renal tissues. EAEAM exhibited minimum IC_{50} values 6.13 ± 1.05, 67.34 ± 1.7, 59.7 ± 3.9, and 49.17 ± 2.19 μg.mL^{-1}. EAEAM (400 mg.kg^{-1}) decreased the creatinine levels from 2.29 ± 0.387 to 0.96 ± 0.095 mg.dL^{-1} and BUN from 92.06 ± 7.949 to 38.18 ± 5.686 mg.dL^{-1} and restored the activities of renal antioxidant enzymes, decreased the lipid peroxidasde (LPO) levels from 158.70 ± 3.542 to 106.91 ± 5.876 nM.g^{-1}, and increased superoxide dismutase (SOD) levels from 12.59 ± 0.463 to 29.95 ± 5.222 U.g^{-1}, glutathione (GSH) from 0.24 ± 0.029 to 0.57 ± 0.048 μM.g^{-1}, and catalase (CAT) from 1.14 ± 0.067 to 3.27 ± 0.296 U.mg^{-1}).

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Antioxidant; cisplatin; glutathione; histopathology; kidney homogenate; lipid peroxidation; nephrotoxicity

Introduction

Cisplatin (CP) is used ubiquitously for the treatment of human malignancies (14). The use of CP is contraindicated because of its nephrotoxic and ototoxic effect (2). Drug-induced nephrotoxicity is a major concern in cases where the patient is sensitive to nephrotoxic drugs. Symptoms of drug-induced nephrotoxicity comprise abnormality in acid–base balance, presence of abnormal content in urine, variation in kidney function test markers, and a slight decrease in glomerular filtration rate (26).

Several mechanisms have been suggested for CP-induced renal toxicity, including apoptosis, inflammatory mechanism, and generation of reactive oxygen species (43). Reactive oxygen species (ROS), such as superoxide anion, hydroxyl radical, and hydrogen peroxide, exhibit beneficial effects on cellular responses and immune function at moderate concentration, but at high levels, ROS can

CONTACT Swapnil Sharma skspharmacology@gmail.com Department of Pharmacy, Banasthali University, Banasthali, Rajasthan 304022, India.

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produce cell injury including peroxidation of membrane lipids, protein denaturation, and DNA damage (31, 36). Antioxidants are considered a good alternative for avoiding or protecting kidneys against nephrotoxicity associated with the use of cisplatin. Several investigators have enunciated the nephroprotective potential of various plant extracts and have demonstrated the role of antioxidants in providing protection against cisplatin induced nephrotoxicity (1, 10, 17).

Substances derived from medicinal plants provide safe and effective antitumor substances with less severe side effects (20, 45). *Aegle marmelos* (Rutaceae) is widely distributed in plains and hills of the Indian subcontinent and South East Asian countries. *A. marmelos* is endowed with various bioactive chemical components including alkaloids, carotenes, phenols, coumarins, flavones, and terpenes, which possibly contribute to its uses in traditional medicine. Fruits of *A. marmelos* are used extensively in traditional medicine for liver disorder, constipation, diarrhea, abdominal distress, hyperthermia, respiratory problems, nausea, sores, swelling, thirst, hiccups, sinusitis, bronchitis, inflammation and edema, urinary retention, and diabetic nephropathy (4, 11). Kore et al. (2011) have established the protective role of A. *marmelos* leaves against gentamicinand doxorubicin induced nephrotoxicity (19). The leaves of A. marmelos are a folk remedy to treat kidney problems viz. urinary retention and diabetic nephropathy (3, 22, 24). Therefore, we explored the antioxidant and nephroprotective potential of A. *marmelos* leaves.

Materials and methods

Chemicals and drugs

2,2-Diphenylpicrylhydrazyl (DPPH), deoxyribose, thiobarbituric acid (TBA), trichloroacetic acid (TCA), nicotinamide adenine dinucleotide (NADH), cisplatin (CP), phenazine methosulfate, nitroblue tetrazolium (NBT), malonaldehyde (MDA), and 5,5-dithio bis-2-nitrobenzoic acid (DTNB) were procured from Sigma Aldrich, USA. Diagnostic kits for creatinine and blood urea nitrogen (BUN) were procured from Span Diagnostic, India; all other chemicals were of analytical grade.

Collection of plant material and extraction

A. marmelos leaves were collected from the Bhopal (M.P., India) region in August–October 2012, and authenticated at the Department of Botany (voucher number 442/Bot/Saifia/13). First, 250 g of leaves were packed in an air-tight container for 5 d with petroleum ether (40:60) with regular shaking at intervals. After 5 d, the solvent was filtered under vacuum, washed again with petroleum ether (40:60), and dried at room temperature. After defatting, the filtrate was successively extracted with petroleum ether, chloroform, ethyl acetate, and 70%

ethanol in a Soxhlet apparatus, and concentrated in rotary vacuum evaporator (Buchi type) at room temperature and weighed; the leaf extracts were termed extracts in petroleum ether (PEEAM), chloroform (CEAM), ethyl acetate (EAEAM), and ethanol (HAEAM), respectively.

In vitro antioxidant assay

In vitro antioxidant activities of the extracts of *A. marmelos* were determined by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, hydrogen peroxide, hydroxyl radical, and superoxide scavenging assays.

DPPH radical scavenging assay

The test samples, ranging from 1 to 20 $\mu g.mL^{-1}$ (2 mL), were put into test tubes containing 2 mL of 1 mM DPPH solution, covered with parafilm, and incubated in the dark for 1 h. Absorbance was noted at 515 nm and IC_{50} was determined (6, 5, 9, 44).

Hydrogen peroxide scavenging assay

Test samples ranging from 10 to 100 $\mu g.mL^{-1}$ (2 mL) were mixed with 1 mL of hydrogen peroxide (20 mM) and incubated for 10 min at room temperature. Absorbance was noted at 230 nm and IC_{50} was calculated (12, 34).

Hydroxyl radical scavenging assay

Reacting mixture was prepared using 100 μL deoxyribose, 50 μL $FeCl_3$, 50 μL EDTA, 100 μL H_2O_2, 550 μL PBS, and 100 μL ascorbic acid. Test samples ranging from 10 to 100 $\mu g.mL^{-1}$ were added to the above reacting mix and incubated at 37°C for 1 h. After incubation, 0.5 mL of *tert*-butanol (TBA) and trichloroacetic acid (TCA) were added. The mix was further incubated for 20 min in a boiling-water bath, cooled at room temperature, and the absorbance noted at 532 nm by UV-VIS spectrophotometty. The test tube with phosphate-buffered saline (PBS) was considered as blank, dimethyl sulfoxide (DMSO) as positive control, and IC_{50} was estimated (13).

Superoxide scavenging assay

Superoxide scavenging was determined using the nitroblue tetrazolium (NBT) reduction method. The reaction mixture consisted of 1 mL of NBT solution (l M NBT in 100 mM phosphate buffer, pH 7.4), 1 mL nicotinamide adenine dinucleotide (NADH) solution (l M NADH in 100 mM phosphate buffer, pH 7.4), and 0.1 mL of test compounds 10–100 $\mu g.mL^{-1}$ and ascorbic acid (50 mM phosphate

buffer, pH 7.4) was mixed. The reaction was started by adding 100 μL of *para*-methyl styrene (PMS) solution (60 μM PMS in 100 mM phosphate buffer, pH 7.4) in the mixture. The tubes were uniformly illuminated with an incandescent visible light for 15 min and the optical density was measured at 530 nm before and after illumination. The percentage inhibition of superoxide generation was evaluated by comparing the absorbance values of the control and experimental tubes, and IC_{50} was determined (29).

Estimation of total phenolic and flavonoid content

The total phenolic contents in the extracts was estimated by the Folin-Ciocalteu method (35). Absorbances were measured at 765 nm and a standard curve based on gallic acid was used for conversion of total phenol concentration to gallic acid equivalent (GAE). The flavonoid content of extracts was determined by a colorimetric assay. Absorbances were measured at 510 nm and a standard curve based on rutin was used for conversion of total flavonoid concentration to rutin equivalent per gram of dry extract (46).

Animals

Animals were selected at random from the animal house of Pinnacle Biomedical Research Institute (PBRI), Bhopal, India, randomly divided into treatment groups, and kept in propylene cages with sterile husks as bedding. They were housed under standard laboratory conditions with relative humidity of 50.7% at 25 ± 2°C and 12:12 light:dark cycle. Animals had free access to standard pellet chow (Golden Feeds, New Delhi, India), and water was available *ad libitum*. All animal experiments were performed with prior permission from the Institutional Animal Ethics Committee (IAEC) of PBRI, Bhopal (CPCSEA Reg No. 1283/PO/c/09/CPCSEA).

Acute oral toxicity

Acute oral toxicity studies were performed in Wistar albino rats as per OECD 423 guidelines (16).

Experimental design (*in vivo*)

The effects of the vehicle, standard, and extracts were screened on CP-induced nephrotoxicity model Wistar albino rats by administering the extracts (EAEAM and HAEAM) and the standard drug. Nephrotoxicity was induced by a single intraperitoneal (i.p.) injection of CP (6 $mg.kg^{-1}$ b.w.). The first groups were the control group (Group-I), the CP control group (Group-II), and the standard group (Group-III). Groups IV and VI were administered with extracts as

described for 5 d: Albino Wistar rats weighing 200 ± 30 g of either sex were randomly selected and divided into six groups of six animals each. Group I (control group), administered 0.1% carboxy methyl cellulose (CMC) orally, 5 $mL.kg^{-1}$, as vehicle served as untreated control, Group II (CP group) received a single intraperitoneal injection of CP (5 $mg.kg^{-1}$ b.w.) and was left for 5 d, Groups III and IV received a daily oral dose of EAEAM at low and high dose, respectively, for 5 d before a single intraperitoneal injection of CP, and Groups V and VI received the HAEAM at low and high dose extract for 5 d following a single intraperitoneal injection of CP.

The route of administration was oral for vehicle and extracts, and intraperitoneal for CP; 0.1% CMC was used as vehicle for extracts and saline for CP. After a single administration CP, rats were sacrificed under light ether anesthesia. Blood samples were collected from tail veins of rats for the estimation of BUN and creatinine. Rat kidneys were removed and processed for the preparation of homogenates for biochemical assay and histological studies.

Biochemical assay for enzymes involved in oxidative stress

Rat kidney homogenates (1 g tissue per 10 volumes of buffer) were prepared in ice-cold buffer (0.1 M Tris-EDTA buffer, pH 7.4) using a homogenizer. The resulting 10% homogenate was centrifuged at 15,000 rpm for 15 min (4°C), and the supernatant(s) obtained was used for the estimation of catalase (CAT), superoxide (SOD), lipid peroxidase (LPO), and glutathione (GSH).

Catalase

Determination of catalase activity was carried out using Sinha's, (1972) method (42). The reaction mixture consisted of 1.96 mL phosphate buffer (0.01 M, pH 7.0), 1.0 mL hydrogen peroxide (0.2 M) and 0.04 mL homogenate (10%) in a final volume of 3 mL, 2 mL dichromate acetic acid reagent was added in 1 mL of reaction mixture, boiled for 10 min and cooled. Changes in absorbance were recorded at 570 nm.

Superoxide dismutase (SOD)

The amount of SOD in the cell-free supernatant was measured (18). Briefly, 1.3 mL of solution A (0.1 mM EDTA containing 50 mM Na_2CO_3, pH 10.5), 0.5 mL of solution B (90 mm NBT- nitroblue tetrazolium dye), 0.1 mL of solution C (0.6% Triton X-100 in solution A), and 0.1 mL of solution D (20 mM hydroxylamine hydrochloride, pH 6.0) were mixed and the rate of NBT reduction was recorded at 560 nm.

Lipid peroxidation (LPO)

LPO was determined by estimating malondialdehyde (MDA) using thiobarbituric acid–reactive substances (TBARS) (27). 10% (w/v) tissue homogenate (0.5 mL)

was mixed with a solution containing 0.2 mL of 80 $g.L^{-1}$ sodium dodecyl sulfate, 1.5 mL of 200 $g.L^{-1}$ acetate buffer (pH 3.5), and 1.5 mL of 8 $g.L^{-1}$ aqueous solution of thiobarbituric acid (TBA). After heating at 95°C for 60 min, upon cooling the red pigment produced was extracted with 5 mL *n*-butanol: pyridine (15:1) mixture. The mixture was vortexed for 1 min and centrifuged for 30 min at 3,000 rpm and the absorbance of the supernatant was measured at 532 nm and expressed as $nM.mg^{-1}$ tissue.

Glutathione (GSH)

Glutathione was measured as according to the method of Ellman (8). To 0.1 mL homogenate, 1.9 mL of 5% TCA (trichloro acetic acid) was added and centrifuged at 3,000 rpm for 15 min to separate the protein. Then 2 mL of 5,5′-dithio bis(2-nitrobenzoic acid) (DTNB) (1 mM) in 0.2 M phosphate buffer (pH 8) was added to 1 mL of supernatant, and absorbance of yellow color was read at 412 nm. The concentration of glutathione was expressed as $nM.mg^{-1}$ tissue.

Kidney function test

Blood samples were centrifuged at 2,000 $rev.min^{-1}$ and the serum was used for estimation of BUN and creatinine using a Biochemistry Auto Analyzer (Star 21 Plus, Chennai, India) (27).

Histological examination

Removed kidneys were fixed in 10% neutral buffered formalin (pH 6.8). Samples from each specimen were sectioned at 50 μm in thickness using a rotary microtome. The sections were stained with hematoxylene and eosin and observed under the microscope (Olympus CH20*i*) at 40× and 100× magnification (41).

Statistical interpretation

All data were calculated as mean ± SD. The results were analyzed and interpreted using one-way ANOVA followed by Bonferroni's test ($P < 0.01$).

Results

The extracts of *A. marmelos* leaves were prepared using petroleum ether, chloroform ethyl acetate, and 70% ethanol, termed PEEAM, CEAM, EAEAM, and HAEAM, respectively. The extractive yields were 3.45% for petroleum ether, 8.67% for chloroform, 13.32% for ethyl acetate, and 18.56% for the hydroalcoholic extract.

The extracts with good antioxidant activity were selected for *in vivo* investigation. Nephroprotective potential of extracts was confirmed on the basis of their influence on kidney function markers and enzymes involved in oxidative stress.

Antioxidant activity

DPPH scavenging activity

All the extracts of *A. marmelos* had DPPH scavenging activities (Fig. 1). The extracts in ethyl acetate (EAEAM) had the highest scavenging activity with lowest IC_{50} value, 6.13 ± 1.05 $\mu g.mL^{-1}$, followed by the extracts in 70% ethanol (HAEAM), petroleum ether (PEEAM), and chloroform (CFEAM).

Hydrogen peroxide scavenging activity

Hydrogen peroxide acts as a precursor to hydroxyl radicals ($^{\bullet}OH$) free radicals, which react with biomolecules to precipitate cellular toxicity (Fig. 1). EAEAM showed the highest hydrogen peroxide scavenging activity with lowest IC_{50} value (70.13 ± 1.5 $\mu g.mL^{-1}$) and was comparable to ascorbic acid (IC_{50} 58.5 ± 3.9 $\mu g.mL^{-1}$), followed by HAEAM, PEEAM, and CFEAM.

Hydroxyl radical scavenging activity

The $^{\bullet}OH$ radical is very toxic, and when it combines with nucleotides in DNA, it causes strand breakage leading to carcinogenesis, mutagenesis, and cytotoxicity. EAEAM was the most effective (59.7 ± 3.9 $\mu g.mL^{-1}$) for hydroxyl radical scavenging activity, followed by HAEAM (74.3 ± 2.6 $\mu g.mL^{-1}$), CFEAM (84.0 ±

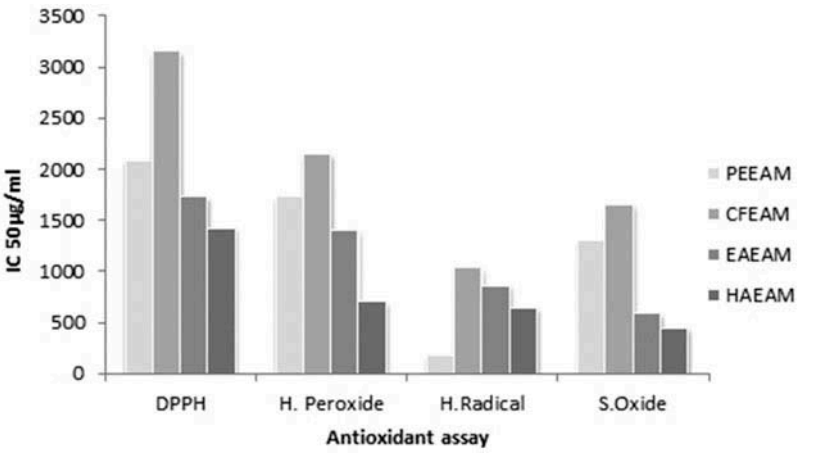

Figure 1. Comparative antioxidant activities of PEEAM, CFEAM, EAEAM and HAEAM in different antioxidant assays.

2.7 $\mu g.mL^{-1}$), and PEEAM (98.3 ± 3.02 $\mu g.mL^{-1}$). The scavenging activity obtained for ascorbic acid was 53.07 ± 2.97 $\mu g.mL^{-1}$ (Fig. 1).

Superoxide radical scavenging activity

Superoxide ($O_2^{\bullet -}$) radical is one of the important reactive oxygen species responsible for the pathogenesis of various diseases. EAEAM (49.17 ± 2.19 $\mu g.mL^{-1}$) demonstrated the most potent superoxide scavenging activity, followed by HAEAM (67.5 ± 2.48 $\mu g.mL^{-1}$) (Fig. 1).

Since the *in vitro* antioxidant studies (Fig. 1) revealed that EAEAM precipitated better antioxidant activity, which was followed by HAEAM, these two extracts were screened for phenolic and flavonoid content estimation and *in vivo* studies.

Total phenolic and flavonoid content

Total phenolic content in *A. marmelos* extracts was 158.64 and 162.00 $mg.g^{-1}$ gallic acid equivalent for EAEAM and HAEAM, respectively, and total flavonoid content was 102.25 and 93.38 $mg.g^{-1}$ rutin equivalent, respectively.

Acute oral toxicity

EAEAM and HAEAM extracts were administered in different groups of albino rats at graded doses from 5 to 2,000 $mg.kg^{-1}$, and no sign of toxicity or mortality was observed up to 2,000 $mg.kg^{-1}$. Therefore, 2,000 $mg.kg^{-1}$ was considered as maximum tolerated dose, and 200- and 400-$mg.kg^{-1}$ doses were selected for *in vivo* investigation in the present study.

Biochemical assay for enzymes involved in oxidative stress

In animals of group II, the level of LPO was higher (158.70 ± 3.542 $nM.g^{-1}$) and the level of SOD, GSH, and catalase were lower than in group I animals. These observations confirmed that CP administration caused an increase in oxidative stress in kidneys. Administration of EAEAM (400 $mg.kg^{-1}$) reduced the LPO level to 106.91 ± 5.876 $nM.g^{-1}$ and elevated SOD, GSH, and catalase level to values of 29.95 ± 5.222 $U.g^{-1}$, 0.57 ± 0.048 $\mu M.g^{-1}$, and 3.27 ± 0.296 $U.mg^{-1}$ compared to the control group. Supplementation with HAEAM, 400 $mg.kg^{-1}$, also precipitated comparative results to that of EAEAM. At lower concentration (200 $mg.kg^{-1}$), HAEAM did not have an effect (Table 1).

Kidney function test

In group II, the level of creatinine and BUN were high compared to vehicle-treated animals, which confirmed the deleterious effect of CP on kidney function. EAEAM and HAEAM decreased the level of creatinine and BUN

Table 1. Effect of *Aegle marmelos* leaf extracts in ethyl acetate (EAEAM) and 70% ethanol (HAEAM) on oxidative stress induced by cisplatin (CP) in albino Wistar rat kidney.

Group	Treatment	Enzymes involved in oxidative stress in kidney[§]			
		LPO ($Nm.g^{-1}$)	SOD ($U.g^{-1}$)	GSH ($\mu M.g^{-1}$)	Catalase ($U.mg^{-1}$)
I	Vehicle only (5 $mL.kg^{-1}$ p.o.)	89.83 ± 2.617	33.95 ± 1.078	0.62 ± 0.057	3.53 ± 0.288
II	Vehicle (5 $mL.kg^{-1}$ p.o.) + CP (6 $mg.kg^{-1}$ i.p.)	158.70 ± 3.542*	12.59 ± 0.463*	0.24 ± 0.029*	1.14 ± 0.067*
III	EAEAM (200 $mg.kg^{-1}$, p.o.) + CP (6 $mg.kg^{-1}$ i.p.)	131.80 ± 9.514**	23.33 ± 3.617**	0.44 ± 0.082**	2.4 9 ± 0.330**
IV	EAEAM (400 $mg.kg^{-1}$, p.o.) + CP (6 $mg.kg^{-1}$ i.p.)	106.91 ±5.876**	29.95 ± 5.222**	0.57 ± 0.048**	3.27 ± 0.296**
V	HAEAM (200 $mg.kg^{-1}$, p.o.) + CP (6 $mg.kg^{-1}$ i.p.)	149.11 ± 5.574	17.02 ± 2.016	0.32 ± 0.027	1.41 ± 0.127
VI	HAEAM (400 $mg.kg^{-1}$, p.o.) + CP (6 $mg.kg^{-1}$ i.p.)	117.93 ± 7.221**	27.48 ± 4.151**	0.52 ± 0.030**	3.03 ± 0.314**

[§]Data presented as mean ± SD ($N = 6$).
*Different from vehicle-treated group ($P < 0.01$). **Different from CP-treated group ($P < 0.001$).

0.96 ± 0.095, 38.18 ± 5.686, 1.11 ± 0.222, and 49.95 ± 7.857 mg.dL^{-1} (P < 0.01) at 400 mg.kg^{-1}. The effect of EAEAM at both doses was effective on the level of creatinine and BUN, but HAEAM was comparatively less effective and precipitated effect only at 400 mg.kg^{-1} (Table 2).

Histological examination

In group I animals, the kidneys showed normal glomeruli, Bowman's capsule, urinary space, podocytes, distal tubules, convoluted tubules, and associated structures. In group II animals, inflammatory infiltration along with apoptotic bodies, degeneration of glomeruli with constriction in urinary space, and cell necrosis were observed. Among the extracts-treated groups, most protective effect was observed for EAEAM, wherein cellular structure was well preserved with no sign of inflammation at both doses (200 and 400 mg.kg^{-1}). In HAEAM-treated rats, at 400 mg.kg^{-1}, nephron protection was significant, but at a dose of 200 mg.kg^{-1}, signs of inflammation and necrosis were observed (Fig. 2).

Discussion

Nephrotoxicity is one of the most serious and dose-limiting toxicities of cisplatin. Cisplatin causes a marked reduction in renal function characterized by an increase in serum blood urea nitrogen (BUN) and creatinine levels (28, 38). Nephrotoxicity associated with CP administration is the composite result of the transport of CP into renal epithelial cells, injury to nuclear and mitochondrial DNA, activation of a multiple cell death and survival pathways, and initiation of a robust inflammatory response (23). Involvement of oxidative stress in CP-induced nephrotoxicity is well established (33). Hence, it is expected that components rich in antioxidants may be useful against nephrotoxicity of cisplatin. Lipid peroxidation is another proposed mechanism for induction of nephrotoxicity in CP therapy (40).

Biochemically, GSH, SOD, and catalase are considered important intracellular components that play a role in normal physiology of the kidneys. GSH reacts with platinum complexes involved in the generation of lower or nontoxic metabolites

Table 2. Effect of *Aegle marmelos* leaf extracts in ethyl acetate (EAEAM) and 70% ethanol (HAEAM) on kidney function in albino Wistar rat model.

Group	Treatment	Creatinine (mg.dL^{-1})[§]	BUN (mg.dL^{-1})[§]
I	Vehicle only (5 mL.kg^{-1} p.o.)	0.84 ± 0.079	18.50 ± 3.146
II	Vehicle (5 mL.kg^{-1} p.o.) + CP (6 mg.kg^{-1} i.p.)	2.29 ± 0.387*	92.06 ± 7.949*
III	EAEAM (200 mg.kg^{-1},p.o.) + CP (6 mg.kg^{-1} i.p.)	1.36 ± 0.212**	61.54 ± 6.206**
IV	EAEAM (400 mg.kg^{-1},p.o.) + CP (6 mg.kg^{-1} i.p.)	0.96 ± 0.095**	38.18 ± 5.686**
V	HAEAM (200 mg.kg^{-1},p.o.) + CP (6 mg.kg^{-1} i.p.)	1.87 ± 0.293	80.73 ± 4.630
VI	HAEAM (400 mg.kg^{-1},p.o.) + CP (6 mg.kg^{-1} i.p.)	1.11 ± 0.222**	49.95 ± 7.857**

[§]Data presented as mean ± SD (N = 6).
*Different from vehicle-treated group (P < 0.01). **Different from CP-treated group (P < 0.001).

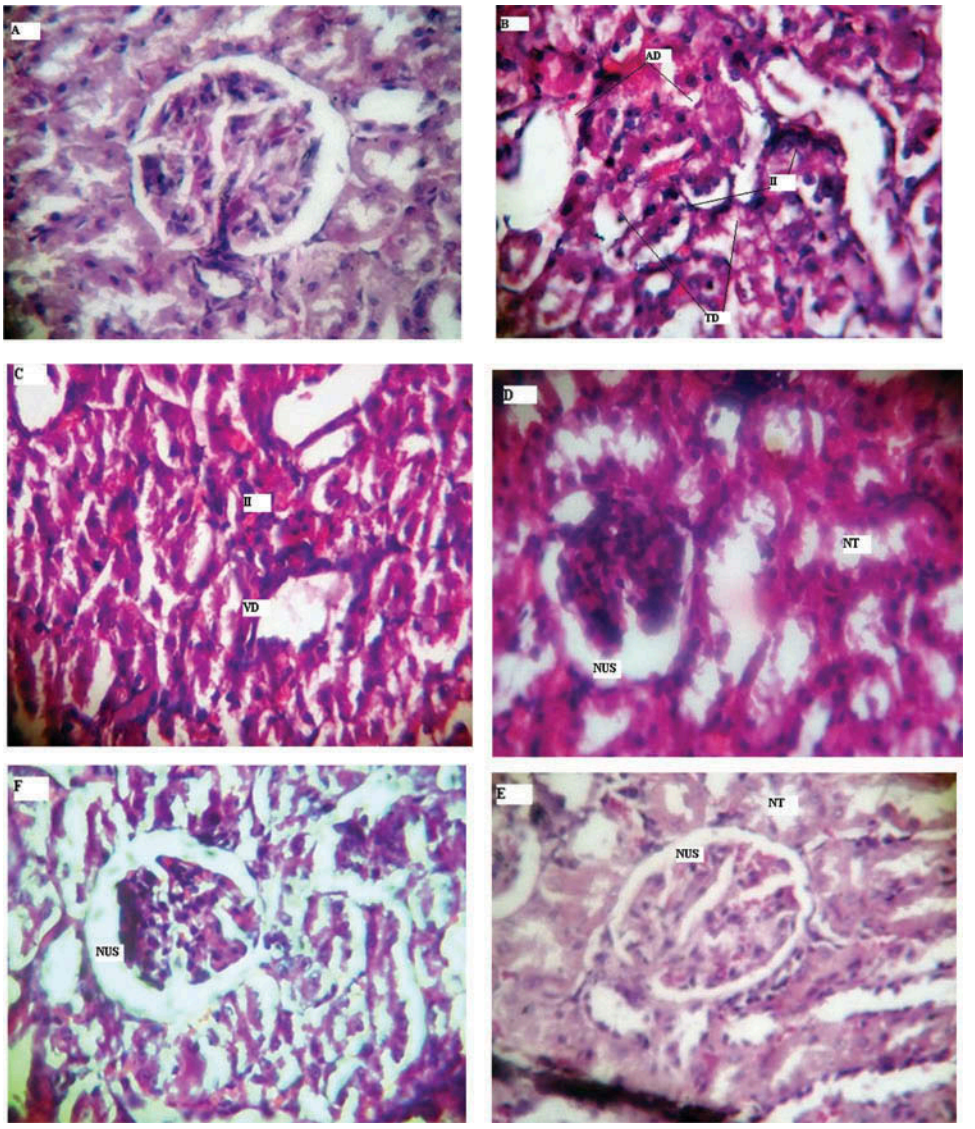

Figure 2. Photograph (40×) of cross section of hematoxylene-and-eosine–stained kidney of: (A) vehicle-treated animal (showing normal architecture of kidney); (B) vehicle + cisplatin (CP)–treated animal (showing architectural damage, inflammatory infiltration, and tubular degeneration); (C) EAEAM (200 mg.kg^{-1}) + CP–treated animal; (D) EAEAM (400 mg.kg^{-1}) + CP–treated animal; (E) HAEAM (200 mg.kg^{-1}) + CP–treated animal; (F) HAEAM (400 mg.kg^{-1}) + CP–treated animal. NUS, normal urinary space; NT, normal tubule; AD, architectural damage; II, inflammatory infiltration; TD, tubular degeneration; EAEAM, ethyl acetate extract of *Aegle marmelos* leaves; HAEAM, 70% ethanol extract of *A. marmelos* leaves.

and hence prevents CP nephrotoxicity. The administration of GSH prior to CP reduces its nephrotoxicity in animal models (37). It has been established that the CP nephrotoxicity is mediated by depletion in GSH concentration and by impaired activities of SOD, and catalase. Antioxidants such as N,N″-diphenyl-*p*-

phenylene-diamine (DPPD), promethazine, ascorbic acid, and GSH provide protection to kidney tissues intoxicated with CP by lowering the index of lipid peroxidation (37). It has been suggested that the availability of SOD at renal proximal tubule cells permitted the administration of high doses of CP and related anticancer agents without causing renal injury (15, 25, 39). Catalase and its derivatives inhibit nephrotoxicity due to CP administration (21). The superoxide radical is an important marker associated with cisplatin-induced nephrotoxicity (7).

The findings of the present study indicate that ethyl acetate extract (at both doses, 200 and 400 $mg.kg^{-1}$) and hydroalcoholic extract (at 400 $mg.kg^{-1}$) of *A. marmelos* leaves protected the kidneys of CP-treated animals that may be attributed to elevated SOD, GSH, and catalase levels with marked reduction in LPO level.

Changes in the serum urea and creatinine concentrations strongly suggest impairment of kidney function by CP. Administration of ethyl acetate extract and hydroalcoholic extract of *A. marmelos* leaves decreased the levels of serum urea and creatinine levels.

In the histological studies, sections of kidney showed the presence of vacuolation, proteinaceous casts, desquamation of epithelial cells, and necrosis. Treatment with ethyl acetate extract of *A. marmelos* leaves restored the normal physiological parameters of the CP-treated animals and was found to be more efficacious than HAEAM (Fig. 2).

Interestingly, CP induced toxicity by various mechanisms of renal dysfunction, such as cellular toxicity, vasoconstriction in the renal microvasculature, and proinflammatory effects, by producing free-radical oxidative stress which participated in the decline of antioxidant enzyme levels of the kidney and led to renal failure (30, 32). Hence, the possible mechanism of nephroprotection by *A. marmelos* leaves could be due to its phenolic and flavonoid content.

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ORCID

Swapnil Sharma http://orcid.org/0000-0003-2639-7096

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