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Amelioration of cisplatin induced nephrotoxicity by *Phyla nodiflora* (L.) Greene

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Phyla nodiflora (Verbenaceae), commonly called frog fruit and locally, *jal bhuti*, is the traditional folk medicine accepted in the Indian Medicine as well as the Traditional system of Chinese Medicine (TCM) for various treatments viz., urinary disorder, lithiasis, knee joint pain, diuresis and swelling. In the present study, we tried to standardise crude *Phyla nodiflora* ethanol extract (PNE) using HPTLC and also evaluated its protection against cisplatin induced nephrotoxicity in rats. HPTLC quantification of rutin and fingerprinting profile was performed. Serum kidney toxicity markers, renal tissue antioxidant and pro-inflammatory cytokine levels were assessed followed by DNA fragmentation assay and histopathological examination of renal tissue. Rutin concentration in ethanol extract was found to be 5.35% w/w. In HPTLC fingerprinting 12 peaks with R_f ranges from 0.08 to 0.86 were confirmed. Serum biochemical parameters, renal tissue antioxidants and pro-inflammatory cytokines levels were found to be restored. The results demonstrated the nephroprotective activity of *P. nodiflora* ethanol extract (400 mg/kg, p.o.) as evidenced by protection of kidneys from cisplatin induced DNA fragmentation and damage tissue architecture.

Keywords: DNA fragmentation assay, Frog fruit, *Jal bhuti*, Nephroprotective, Rutin

Cisplatin (cis-diammine dichloroplatinum II, CDDP) is platinum-based potent antineoplastic drug used in most of the chemotherapy regimens for solid or hematologic tumors. Cisplatin forms inter and intra stand cross links with DNA of the cell and arrest its synthesis and replication in rapidly proliferating tumour cells. Cisplatin-DNA crosslinks cause cytotoxic lesions in tumour cells and other regenerative cells[1]. Among gastro toxicity, myelosuppression, ototoxicity and allergic reaction, nephrotoxicity is the major dose limiting side effects[2]. Approximately, 20% patients under cisplatin chemotherapy suffer severely from renal dysfunction and one third of patients experience acute renal toxicity after few days of initial treatment. The mechanism of cisplatin-induced nephrotoxicity involves many cellular processes including DNA damage, inflammation, oxidative stress and apoptosis. Cisplatin preferentially accumulated within the proximal tubule cells in the outer medulla of the kidney. It lowers the antioxidant enzymes leading to enhanced generation of reactive oxygen metabolites (ROM) and lipid peroxidation, and thereby plays an important role in cisplatin-induced renal cell injury[3]. It has been reported that cisplatin induces release of proinflammatory cytokines like TNF-α, IL1β and IL6 by renal epithelial cells. TNF-α causes oxidant stress by sensitizing infiltrating leukocytes[4-7]. There is no specific medicine for renal toxicity under chemotherapy. Only supportive care is provided for renal function restoration like hydration with normal saline, use of corticosteroids for reduction of inflammation or lowering the dose of drug causing nephrotoxicity[8]. However, herbal remedy is consistently gaining acceptance for the treatment of acute renal failure.

Many Indian medicinal plants are effective against acute renal injury[9]. *Phyla nodiflora* (L.) Greene (Syn. *Lippia nodiflora* Greene or *Lippia nodiflora* Michx) belonging to the Fam. Verbenaceae is the promising folk medicinal herb. This herbaceous, low-growing, perennial plant creeps on the ground and spreads quickly over ground in tropical to warm temperate regions of the world[10-12]. In India, it is grown commonly in Dindigul, Pudhukottai, Nagapattinam, Thoothukudi and Coimbatore districts of Tamil Nadu. Traditionally, it is used to treat urinary disorders, gonorrhoea, menstrual disorders and respiratory diseases. It has been reported to possess antispasmodic, antimalarial, analgesic, anti-inflammatory, antipyretic, diuretic, antimicrobial, antidiabetic activity, hepatoprotective, antitumor,

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urolithiasis [13-19]. The whole plant extract is reported to induce apoptosis and cell cycle arrest in human breast cancer cell line, MCF-7[20]. It contains a variety of phytoconstituents such as triterpenoids, flavonoids, phenols and steroids. Some of the chemical constituents which were determined from GC-MS analysis were 2,7-dioxatricyclo[4.3.1.0 (3,8)]decan-4-one, stigmasterol, benzoic acid, 4-etoxy-, ethyl ester, azacyclotridecan-2-one and n-hexa-decanoic acid[21]. In this study, we made an attempt to carry out phytochemical standardisation of the crude ethanol extract of *Phyla nodiflora* using HPTLC, and also explored its nephroprotective activity against the cisplatin-induced renal damage in rats.

Materials and Methods

Plant authentication and Extraction procedure

Whole plant of *Phyla nodiflora* was collected from Dindigul, Tamil Nadu and authenticated by Prof. NK Dubey, Department of Botany (Banaras Hindu University), Varanasi. The voucher specimen (Cog/PN/2014-15) was deposited in the Department of Pharmaceutics, Indian Institute of Technology (Banaras Hindu University), Varanasi (UP) for future references. The fresh plants were cleaned by washing in running tap water then dried under shade for seven days. Dried plants were coarsely powdered using a mechanical grinder and sieve no. 60. About 500 g of coarsely powdered drug was exhaustively extracted in a soxhlet extractor using 95% ethanol for three days. The extract obtained was concentrated using vacuum rotary evaporator (IKA RV 10, China) to yield the crude *Phyla nodiflora* ethanol extract (PNE).

Phytochemical evaluation

The dried crude extract was qualitatively standardised by preliminary phytochemical screening to detect the presence of various phytochemical classes. The total of phenolic content, tannin content, flavonoids, and flavonols was done by spectrophotometric quantification method and all the results were carried out in triplicates[22].

High performance thin layer chromatography analysis

Rutin was purchased from Natural remedies Pvt. Ltd. Bengaluru. Merk $60F_{254}$ (E. Merck) silica plates of uniform thickness of 0.2 mm were used for plate development. The analysis was done by using Camag–HPTLC instrumentation equipped with Linomat V sample applicator and WINCATS 4 software for data interpretation, Camag TLC scanner 3, Camag TLC visualizer. The Rf values were recorded and the developed plate was analyzed and photodocumented at ultraviolet range with wavelength (λmax) of 254 nm. Sample was prepared in methanol at different concentration. PNE was chemically standardized by using rutin (flavonoid glycoside) as standard, using solvent system ethyl acetate: formic acid: acetic acid: water (20:2.2:2.2:1.1) as solvent system. The HPTLC fingerprinting was done using mobile phase toluene: ethyl acetate: formic acid (10:3:1).

Experimental animals

The certified pathogen free healthy Charles Foster albino male rats (150-250 g) were procured from the Central Animal House (Reg. No. 542/02/ab/CPCSEA), Institute of Medical Sciences (Banaras Hindu University), Varanasi, India. Animals were housed in polypropylene cages and maintained under standard conditions (12 h light and dark cycles at an ambient temperature of 25°□C and 45-55% RH). The animals were allowed to acclimatize to the environment for 7 days before the commencement of experiments. They were fed with commercially available standard rat feed and water *ad libitum*. All experimental protocols were approved from Central Animal Ethical Committee of Banaras Hindu University (No. Dean/2015/CAEC/1132) and were conducted in accordance with accepted standard guidelines of National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication no. 85–23, revised 1985).

Optimisation of cisplatin dose to induce nephrotoxicity

To evaluate the dose of cisplatin which induce nephrotoxicity, rats were randomly divided into three groups with six animals each. Cisplatin (Cytoplatin-10, cipla), was given at different dose of 4 mg/kg, i.p., 6 mg/kg, i.p., and 8 mg/kg i.p., after 72 h of cisplatin administration blood was collected through retro-orbital venous plexus under light anaesthesia then serum was separated and tested for urea and creatinine level. Serum urea kit and creatinine kit were purchased from Erba Diagnostics Mannheim, Germany.

Experimental setup and drug treatment protocol

In order to evaluate the effect of PNE on cisplatin induced nephrotoxicity, rats were randomly divided into five groups with six animals in each. The treatment details of groups were as follows: Group I (Normal control): vehicle (aqueous solution of 0.5% CMC) for 7 consecutive days and 0.9% NaCl on 4th day; Group II (Toxic control): vehicle (aqueous solution of 0.5% CMC) for 7 consecutive days and

cisplatin (6 mg/kg, i.p.) on 4th day; and Groups III-V: PNE (@100, 200 and 400 mg/kg, p.o.) for 7 consecutive days and cisplatin (6 mg/kg, i.p.) on 4th day, respectively. The PNE extract was suspended in 0.5% CMC and given orally. PNE and vehicle were given orally for total 7 days, cisplatin (6 mg/kg, i.p.) freshly prepared in normal saline (0.9% NaCl) was administered to respective rats on 4th day of the treatment. Finally on 7th day, animals were sacrificed to collect blood and kidney for various biochemical tests, antioxidant parameters, estimation of pro-inflammatory cytokines level and histological studies.

Biochemical parameters in serum

Serum urea and serum creatinine level were measured by autoanalyzer using BUN method and modified rate Jaffe's kinetic method. Serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), total protein, total bilirubin and direct bilirubin and alkaline phosphate (ALP) level were analysed according to standard protocol given in enzyme assay kit (Span diagnostic Ltd) using auto analyser[23].

Antioxidant parameters in kidney tissue homogenate

Minced kidney tissues were homogenized in 0.1 M potassium phosphate buffer (pH 7) in a tissue homogenizer containing protease inhibitor to get 10% homogenate (w/v), which was centrifuged at 10000×g, for 20 min at 4ºC. The supernatant was utilized for antioxidant status. Lipid peroxidation (LPO) levels were determined in terms of thiobarbituric acid reacting substance (TBARS) and expressed as equivalent to malondialdehyde (MDA) using 1'1'3'3'-tetramethoxypropane as standard malondialdehyde (MDA). Superoxide dismutase (SOD) was estimated in terms of its capacity to inhibit the reduction of nitro blue tetrazolium (NBT) by superoxide, generated in the presence of riboflavin in reaction system through a photosensitive reaction. Catalase (CAT) activity was expressed from the rate of decomposition of H_2O_2 at 240 nm following the addition of tissue homogenate. Reduced glutathione (GSH) level was calculated as protein-free sulfhydryl content using 5,5-dithiobis-2-nitrobenzoic acid (DTNB).

Proinflammatory cytokines in kidney tissue homogenate

The proinflammatory molecules generated by cisplatin induced nephrotoxicity were investigated by measuring cytokine levels of TNF-α, IL-6 and IL-1β on homogenised kidney tissues using ELISA kits for determination of interleukin-1β, interleukin-6, TNF-α (Komabiotech, Korea). Given standard and detection antibodies needed to be reconstituted in sterile water. Standard and samples were diluted in serial dilution. Wells were selected in microplate and washed with washing solution then 100 mL of standard and samples were added to wells followed by addition of diluted detection antibody after incubation. Colour was developed after addition of colour development enzyme and colour development solution. Finally, absorbance was measured by microplate reader (BioTek instruments Inc. USA).

DNA fragmentation assay by agarose gel electrophoresis

Kidneys were harvested and washed with sterile water and cut into small pieces using sterile surgical blade followed with homogenisation in PBS buffer. Homogenate was centrifuged for three times to remove cell debris and RBCs at 3000 rpm for 10 min at 4°C, and the supernatant was discarded. The pellet of cells was washed twice with PBS and lysed in a buffer containing 50 mmol/L Tris–HCl pH 8.0 and 0.5% SDS, and incubated at 37°C for 30 min then mixed uniformly. The pellet was incubated with 1.0 µL of DNAase free RNase (10 mg/mL) for 1 h at 37°C. Further, proteinase K (50 µg/mL) was added and incubated for 90 min at 50°C. The precipitated DNA was dissolved in a 5 µL of Tris-EDTA buffer and quantified spectrophotometrically. An equal concentration of DNA (10 µg) stained with ethidium bromide had been resolved on a 1% agarose gel at 50 V for 4 h, viewed under UV light, and documented using the Alpha Innotech system (San Leandro, California, USA).

Histopathological study

Kidneys were dissected out and after washing with isotonic saline were fixed in 10% neutral buffered formalin for 48 h and embedded in paraffin wax. Paraffin blocks of the tissues were sectioned (5-6 µm thickness) by microtome then stained with Hematoxylin and Eosin (H&E) and subjected to the microscopic and imaging system (Nikon, Japan).

Statistical analysis

The experimental data are expressed as mean ± S.E.M., with six animals in each group. The analysis of variance was performed with Graph pad Prism 5.0 software (Graph Pad Software, Inc., La Jolla, CA) followed by one-way ANOVA. Dunnett's Comparison test was applied for determining the statistical significance between different groups.

Results

Phytochemical evaluation

The yield of PNE was found to be 16.6% w/w of air dried plant material. The qualitative phytochemical screening of PNE showed the presence of alkaloids, flavonoids, phenolics, phytosterols, tannins, saponins and glycosides. Total flavonoids and flavonols content measurement was done triplicate and was found to be 7.63 mg/g and 3.45 mg/g (rutin equivalent per gram plant material), respectively. The total phenolic content and total tannin content in PNE was found to be 56.15 mg/g and 34.21 mg/g (tannic acid equivalent per gram plant material), respectively.

High performance thin layer chromatography analysis

HPTLC results for quantification of rutin and finger printing was performed at scanning wavelength at 254 nm and chromatogram were obtained. The peak of rutin was identified and compared by comparing their retention factor (Rf) values with that of standards rutin. The quantity of rutin was found to be 5.35% w/w. The study revealed that solvent system toluene:ethyl acetate:formic acid (10:3:1) showed maximum number of peaks in the range of R_f value 0.08 to 0.86 at wavelength 254 nm (Fig. 1). The chromatogram suggested that out of 12 compounds the compounds with R_f value 0.22 and 0.37 were predominant as they had a maximum percentage area with 45.73% and 14.89%. Phytoconstituents with their R_f values, peak height and area are given in (Table 1).

Optimisation of cisplatin dose to induce nephrotoxicity

Rats administered with 4 mg/kg i.p. of cisplatin showed no significant elevation (P >0.05) in serum urea and creatinine concentration after 72 h of cisplatin administration. 6 and 8 mg/kg, i.p. of cisplatin showed marked elevation (P <0.05) in serum urea and creatinine concentration in rats. At 8 mg/kg, i.p. of cisplatin two rats out of six died. Therefore, to evaluate nephroprotective activity of PNE, 6 mg/kg, i.p of cisplatin was used for further induction of nephrotoxicity in rats (Fig. 2).

Effect of PNE on serum biochemical parameters

Renal toxicity was induced by cisplatin (6 mg/kg, i.p.) followed by treatment with PNE at different doses. PNE (400 mg/kg, p.o.) for seven days significantly decreases (P <0.05) the elevated levels of biochemical parameters to normal as compared to control group. PNE (100 and 200 mg/kg, p.o.) did not show significant protection (P >0.05) (Table 2).

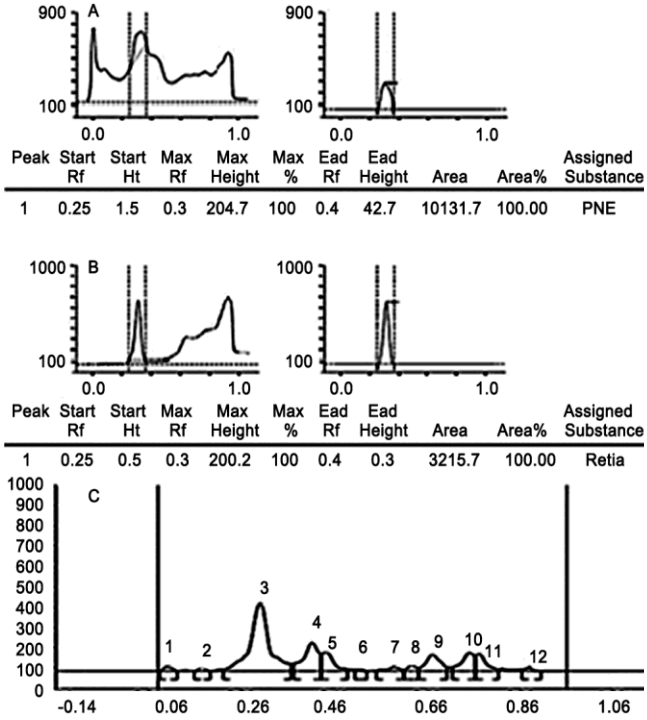

Fig. 1 — HPTLC chromatogram of (A) PNE showing peak of rutin; (B) standard rutin; and (C) HPTLC fingerprinting profile of PNE

Table 1 — HPTLC finger printing profile of PNE showing various peaks

Peak	Start R_f	Max R_f	End R_f	Start height	Max height	End height	Area	Area %
1	0.08	0.10	0.12	1.0	23.8	2.1	369.7	1.50
2	0.16	0.18	0.19	1.2	16.7	1.1	210.6	0.86
3	0.22	0.30	0.37	8.7	340.5	38.0	11261.4	45.73
4	0.37	0.41	0.43	39.4	144.6	87.5	3666.6	14.89
5	0.43	0.44	0.49	87.8	95.7	10.9	1973.2	8.01
6	0.50	0.51	0.53	8.5	12.1	1.1	152.5	0.62
7	0.55	0.59	0.61	3.5	24.2	6.2	482.9	1.96
8	0.61	0.62	0.64	6.8	28.4	18.1	467.8	1.90
9	0.64	0.67	0.70	18.2	85.3	19.8	2129.1	8.65
10	0.71	0.75	0.76	21.1	94.7	78.6	2045.3	8.31
11	0.76	0.77	0.81	79.8	87.8	9.6	1597.2	6.49
12	0.86	0.88	0.91	10.4	23.9	0.2	270.4	1.10

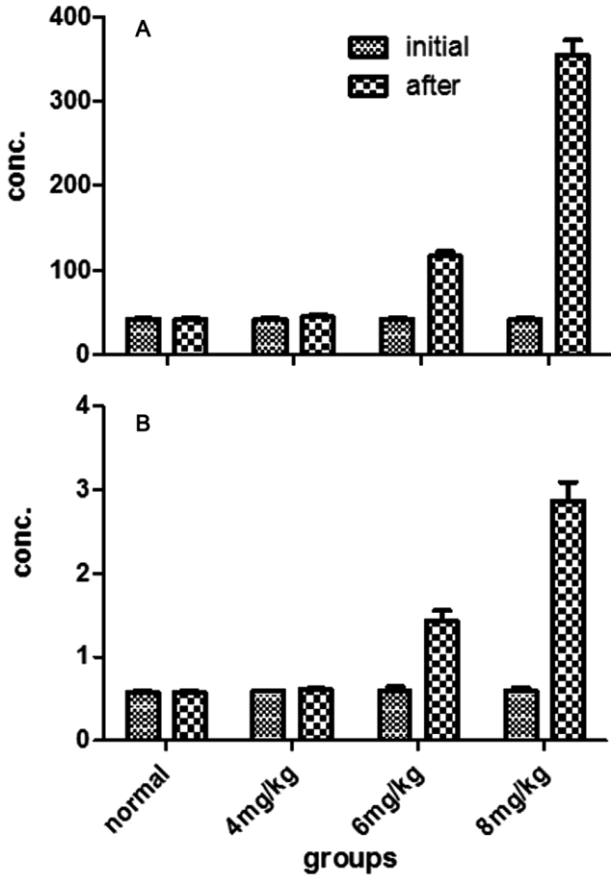

Fig. 2 — Serum Urea and Creatinine concentration at different doses of cisplatin

Effect of PNE on antioxidant status in renal tissues of rats

The activity of SOD, Catalase and GSH content was significantly decreased while MDA level was significantly increased in cisplatin (6 mg/kg, i.p.) treated rats as compared to control group. PNE (400 mg/kg, p.o.) treatment significantly inverted the changes in antioxidant enzymes in dose dependent manner (P <0.05). Treatment with PNE significantly normalised the levels of catalase, GSH and MDA level (Table 2).

Effect of PNE on proinflammatory cytokines in renal tissue of rats

By measuring cytokine levels of TNF-α, IL-6 and IL-1β on homogenised kidney tissues using standards. It was evaluated that there was production of proinflammatory molecules in renal tissue homogenate, due to inflammation caused by cisplatin. However, PNE treated rats (400 mg/kg, p.o.) reduced expression of these proinflammatory molecules in the kidney tissues (Table 2).

DNA fragmentation assay by agarose gel electrophoresis

From the figure it can be observed that cisplatin treatment result in a substantial increase in oligonucleosome-length degradation of DNA. However, the PNE (400 mg/kg, p.o.) treatment significantly inhibited this smear length and there was approximately no smearing occurred, it showed significant protection of DNA damage (Fig. 3).

Table 2 — Effect of various doses of ethanol extract of *Phyla nodiflora* on various parameters in cisplatin treated nephrotoxic rats

Parameters	Unit	Treatment groups					
		Control	Toxic control	PNE 100 mg/kg	PNE 200 mg/kg	PNE 400 mg/kg	Rutin
Biochemical analysis in blood serum							
Urea	(mg/dL)	46.8±0.09	71.9±2.17***	66.05±2.61***	59.51±2.24**	48.71±2.6	50.48±2.24
Creatinine	(mg/dL)	0.39±0.004	0.57±0.05*	0.56±0.03*	0.47±0.04	0.39±0.05	0.46±0.04
SGOT	(IU/L)	137.14±0.67	170.06±3.4***	163.48±5.7**	159.33±4.3**	143.17±6.16	149.5±4.3**
SGPT	(IU/L)	65±4.9	88.05±3.4***	84.41±2.4**	78.7±4.19	63.55±3.6	69.85±4.3
TP	(g/dL)	7.4±0.4	5.95±0.28	5.9±0.68	6.67±0.23	6.95±0.46	7.09±0.33
TB	(mg/dL)	1.24±0.22	4.4±0.37***	4.3±0.26***	2.6±0.46*	1.6±0.50	3.01±0.45**
DB	(mg/dL)	0.41±0.04	1.04±0.012**	0.81±0.15	0.76±0.15	0.55±0.14	0.57±0.15
ALP	(IU/L)	68.4±3.1	258.4±13.9***	254.2±10.1***	152.8±6.09***	77.8±6.5	80.16±4.6*
Antioxidant enzymes							
CAT	(g/mg of protein)	28.45±0.8	12.03±1***	15.09±0.76***	20.88±0.82***	26.96±0.69	25.93±0.55*
LPO	(nmol MDA/g of tissue)	24.58±2.34	61.93±7.9***	59.18±6.23***	32.35±2.25	21.83±3.4	26.32±8.12
SOD	(Units/mg protein)	36.38±0.33	14.53±2.34***	22.35±0.84***	23.69±0.77***	36.42±0.59	33.12±1.94
GSH	(μg glutathione per mg protein)	264.2±2.64	122.2±8.4*	139.3±7.49*	199.15±12.0	259.28±6.3	250.14±8.24
Proinflammatory cytokines							
IL-1β	(pg/mL)	483.7±5.2	1211.78±3.4***	1016.78±5.5***	877.05±6.8***	519.76±15.45*	548.73±9.4***
IL-6	(pg/mL)	723.42±8.17	1271.75±12.33***	1285.083±13.5***	1069.25±9.6***	746.75±10.38	780.08±15.7**
TNF-α	(pg/mL)	169.7±3.2	857.3±3.9***	821.2±2.9***	647.1±10.1***	231.2±24.9**	238.59±22.89**

[Values are mean ± SEM, (n=6). One way ANOVA followed by Dunnett's test, Note: n=6 in each group *P value <0.05, **P value <0.01, ***P value <0.001]

Histopathological study

Renal sections of control group (Gr. I) showed normal histology. Kidneys of cisplatin treated rats (Gr. II) were found to have distorted histology with atrophied glomerulus, extensive loss of tubular epithelial cells, tubular dilation, collecting tubules showing necrosis, intra-tubular debris and cast formation and vacuolization. The treatment group with 100 mg/kg dose of PNE (Gr. IIIA) showed altered histology with no protective effect whereas The treated group with 200 mg/kg dose of PNE (Gr. IIIB) showed damaged histology with less protective effect but treatment group with 400 mg/kg dose of PNE (Gr. IIIC) showed protective action by reducing inflammation, minimizing necrosis and vacuolization (Fig. 4).

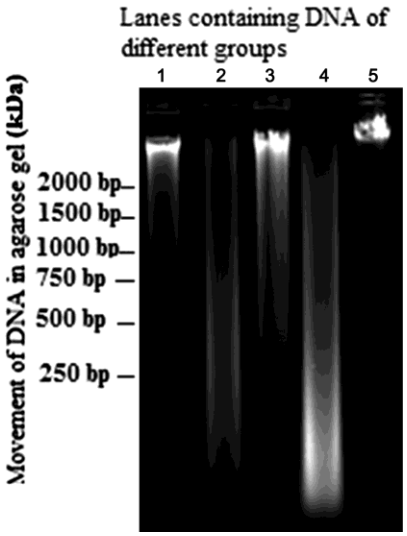

Fig. 3 — Agarose gel electrophoresis of cisplatin and PNE treated rats. [Lane 1: PNE 400 mg/kg, p.o., Lane 2: PNE 200 mg/kg, p.o., Lane 3: PNE 100 mg/kg, p.o., Lane 4: cisplatin 6 mg/kg, i.p., Lane 5: control. Each lane reflecting the presence of DNA fragments was viewed on an ethidium bromide-stained gel]

Discussion

Kidneys help in maintaining the homeostatic balance of body fluids by filtering and secreting toxic metabolites from the blood. They have vital role in controlling blood pressure, erythropoiesis and glucose metabolism. Therefore, to conserve the kidney functions from various toxic agents is foremost. Cisplatin is a potent platinum based anticancer drug. Its major dose limiting side effect is nephrotoxicity, as it accumulates five times more in tubular epithelial cells in comparison to serum. It also causes impairment in liver function and marked elevation in hepatic enzyme. Its mechanism of nephrotoxicity is different from killing tumour cells. The accumulation of cisplatin is highest in S3 segment of PCT of the inner cortex and outer medulla and it is taken up to PCT of nephron through the organic cation transporter (OCT 2) and also through passive diffusion.

Pathogenic events involved in nephrotoxicity are mainly cellular oxidative damage by free radicals and production of various proinflammatory cytokines which leads to oxidative stress and inflammation as consequences[24-28].

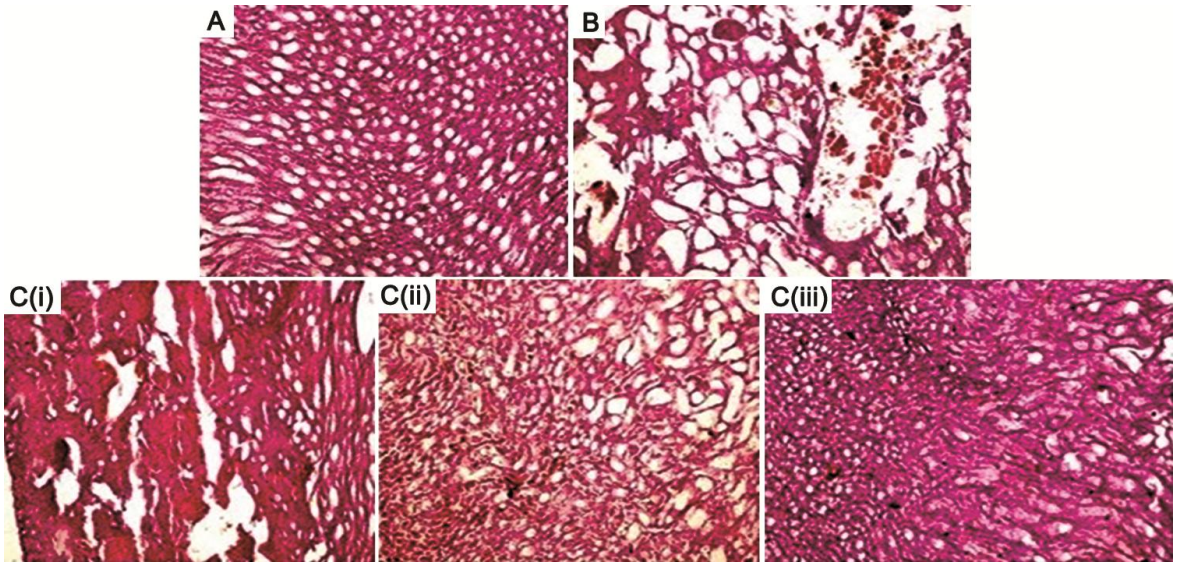

Fig. 4 — Tissue architecture of rat kidney showing the effect of PNE at different doses in cisplatin induced tubular damage. [Gr. I: control, Gr. II: Toxic control group, Gr. IIIA: Treatment group with 100 mg/kg, per oral of PNE, Gr. IIIB: Treatment group with 200 mg/kg, per oral of PNE, Gr. IIIC: Treatment group with 400 mg/kg, per oral of PNE]

In the present study, *Phyla nodiflora* extract (PNE) was chemically standardized with rutin using HPTLC. The flavonoid rutin was taken as the standard for further experiments as it is been reported to have anti-inflammatory, antioxidant and nephroprotective properties[29-31]. The dose standardisation of cisplatin (by taking different doses) evaluated that at a single dose of cisplatin (6 mg/kg, i.p.), produces nephrotoxicity after 72 h of administration. It was evidenced from elevated level of serum urea, creatinine and other biochemical parameters. Study found that of PNE (400 mg/kg, p.o.) lowers the cisplatin induced raised levels of biochemical markers and also restore the antioxidant enzymes like GSH, CAT and SOD which might be due to excessive accumulation ROS by cisplatin. As LPO is an autocatalytic process, the LPO assay is the convenient method to measure oxidative damage to tissues, hence we did. MDA is one of the end products in the lipid peroxidation process and releases during oxidative degeneration as a product of free oxygen radicals, which can be calculated as an indicator of lipid peroxidation. It was found that MDA production was significantly reduced by treatment of PNE.

Inflammation is a major consequence in various pathological conditions. The role of inflammation in nephrotoxicity is highly acknowledged with the participation of proinflammatory cytokines, chemokines, leukocytes and adhesion molecules. TNF-α is an intercellular chemical messenger and is involved in the inflammatory process released by T lymphocytes, white blood cells, macrophages and monocytes. In infection, sepsis, and ischemia IL-1β has been reported to be a proximal mediator of the inflammatory events. IL-6 (polypeptide) secreted from activated macrophages, monocytes, adipocytes, endothelial cells and fibroblasts in response to various stimuli, such as TNF-α, IL-1β, bacterial endotoxins, physical exercise and oxidative stress. In our study, it is demonstrated that there was significant increase in IL-1β, IL-6 and TNF-α in kidney tissues of rats treated with cisplatin. Cytokines IL-6, IL-1β and TNF-α orchestrate the inflammatory response. PNE also prevented oxidative DNA damage in renal tissues in cisplatin treated rats. Normal histological feature indicated in transverse section of kidney tissue was also get impaired including swelling, vacuolization, proximal tubular necrosis, glomerular congestion and inflammation by single dose of cisplatin. Damaged tissue architecture by cisplatin brought to normal by PNE treatment at dose of 400 mg/kg, p.o. It is justified from the present study that PNE (400 mg/kg, p.o.) has protective effect by resisting cisplatin induced nephrotoxicity. The mechanism by which PNE attenuates cisplatin induced nephrotoxicity is suggested to be via depreciation of free radical oxidative stress and proinflammatory cytokines levels.

Conclusion

The observed results of treatments with the crude ethanolic exttact of *Phyla nodiflora*, describe mechanism to attenuate cisplatin induced nephrotoxicity by reducing the oxidative stress and inflammatory markers predominantly IL-1β, IL-6 and TNF-α. It also protected cisplatin administered kidneys from DNA fragmentation and altered tissue architecture. The results suggest that the jal bhuti, *Phyla nodiflora* could be considered as a new source of rutin and also demonostrated its protective effect against cisplatin mediated tubular damage.

Conflict of interest

Authors declare no conflict of interests.

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