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Nephroprotective Evaluation of Ethanolic Extract of the Seeds of Papaya and Pumpkin Fruit in Cisplatin-induced Nephrotoxicity

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Abstract:

The present study is focused on the Nephroprotective evaluation of ethanolic extract of the papaya seed, PaSE (Biological name- *Carica papaya*, family – Caricaceae) and pumpkin seed, PuSE (Biological name - *Curcubita pepo*, family – Curcurbitaceae). Cisplatin (10mg/kg, i.p.) used for the nephrotoxicity, which is the dose limiting side effect of the Cisplatin (Cis-diamine dichloro platinum-II). The ethanolic extract of PaSE & PuSE exhibited protection against cisplatin-induced nephrotoxicity, which were proved by the gross behavioural studies, histopathological, renal function and biochemical studies. Antioxidant studies like nitric oxide scavenging activity, lipid peroxidation in kidney also supporting the nephroprotective activity of these seeds. This nephroprotective study also compared with chloroform extract of the dried Zinger roots, ZE (*Zingiber officinale Rosc,* Family- Zingiberaceae) and methimazole (MZL) which is already evaluated. Histopathological investigation of the kidney like glomerular congestion, blood vessel congestion, intestinal edema, inflammatory cells, necrosis, tubular casts were also observed for control, test and reference groups.

Keywords: nephrotoxicity, cisplatin, seeds of *Carica papaya*, seeds of *Curcubita pepo*, roots of *Zingiber officinale* Rosc.

Introduction:

Nephrotoxicity is a poisonous effect of some substances, both toxic chemicals and medication (nephrotoxins are chemicals displaying nephrotoxicity) on the kidneys. Nephrotoxicity caused by cisplatin probably due to apoptosis, inflammatory mechanism and generation of reactive oxygen species (Dirk TH et.al.,1985,) [1,2]. Hence a systematic pharmacological evaluation of nephroprotective effect of the ethanolic extract of PaSE & PuSE carried against experimentally induced renal damage. The chloroform extract of the roots of the *Zingiber officinale* Rosc (T.A. Ajith et.al., 2007) [3] and the drug methimazole (A.M. Osman et. al., 2000) [4] is already proved for its nephroprotective activity. This study were purposed the evaluation of nephroprotective evaluation of ethanolic extract of PaSE & PuSE with comparing MZL and chloroform extract of *Zingiber officinale* Rosc roots activity.

Our objectives of this study is to find out the natural herbal remedies in such cases where nephroprotectivity can be achieved, which is caused by use of some regularly usable nephrotoxic drugs like ACE inhibitors, angiotensin receptor blockers (arbs), NSAIDS, sulfonamides, amphotericin B, quinolones etc. Studies of renal function & other parameters, lipid peroxidation in kidney, antioxidant profile and histopathological studies in mice treated with ethanolic extract of PaSE & PuSE showed nephroprotective activity significantly (Table 1, 2 & 3).

Materials and Methods:

Drugs and Chemicals: cisplatin and methimazole were obtained as gift sample from Aurobindo Pharma Limited, Plot No.2, Maitri Vihar, Ameerpet, Hyderabad, India. For the evaluation of nephroprotective

activity parameters were estimated by the use of commercial kits and laboratory method. All the chemicals used for the study were purchased either from S.D.fine or Merck (India).

Preparation of the Extract: The seeds of Papaya fruit and pumpkin were collected from the local market and were shade dried followed by drying in hot air oven for 30 min at low temperature, then it were powdered in a mechanical grinder. The dried coarse powder (1kg) were subjected to extraction with 5 litre of ethanol for 48 hrs, the ethanolic extract were collected, filtered and concentrated in vacuum under reduced pressure and moisture dried in dessicator. The yield was found about 0.65% w/w for seeds of Papaya fruit and 0.80 % w/w for seeds of pumpkin fruit. Similarly *Zingiber officinale* Rosc roots also collected from local market and ethanolic extract were prepared.

Preliminary phytochemical analysis: The ethanolic extract of PaSE and PuSE were subjected to preliminary phytochemical analysis to test for presence or absence of various phytoconstiuents. It is found the presence of alkaloids, protein, steroids, tannins, flavanoids, sterols, saponins, terpenoids, phenols. carbohydrates, glycosides, proteins.

Animals: Adult inbred male Swiss albino mice weighing 75-100g and three months old were used for the study. Mice were housed in colony cages of 6 animals per cage and were given chow pellets and water ad libitum and maintained at 25 ± 2^0C and 45-55% relative humidity. Animals were divided into 6 groups, containing 6 animal in each.

Nephroprotective activity-Study protocol:

GroupI(Control)-Animals were administered with equivalent volume of 0.1ml i.p. of normal saline for 9 days.

GroupII(Cisplatin)-Animals were received 10mg/kg/day i.p. of cisplatin for 9 days to induce nephrosis.

GroupIII(Cisplatin+PaSE)-Animals were received 10mg/kg/day i.p. of cisplatin for 9 days to induce nephrosis and 100mg/kg/day i.p. of PaSE from 10^{th} to 19^{th} day of the study.

GroupIV(Cisplatin + PuSE)-Animals were received 10mg/kg/day i.p. of cisplatin for 9 days to induce nephrosis and 100mg/kg/day i.p. of PuSE from 10^{th} to 19^{th} day of the study.

GroupV(Cisplatin+ZE)-Animals were received 10mg/kg/day i.p. of cisplatin for 9 days to induce nephrosis and 100mg/kg/day i.p. of GE from 10^{th} to 19^{th} day of the study.

GroupVI(Cisplatin+MZL)-Animals were received 10mg/kg/day i.p. of cisplatin for 9 days to induce nephrosis and 40mg/kg/day i.p. of MZL from 10^{th} to 19^{th} day of the study.

Acute toxicity and gross behavioural studie[5]: To induce nephrotoxicity cisplatin at a dose of 10mg/kg, i.p. were used. Gross behavioural responses like vocalization on touch, locomotor activity, palpebiral reflex, autonomic responses such as tremors, convulsion, salivation, diarrhoea, sleep, coma were monitored periodically and also mortality observed for 21 days.

Evaluation of renal function[6]: For the Evaluation of renal function parameters were evaluated like creatinine clearance, urinary protein (sulphosalicylic acid method), serum total proteins (S_{TP}: biuret method), urine to serum creatine ratio, body weight change (%), serum creatinine (alkaline picrate method), serum total protein, serum uric acid, blood urea nitrogen (diacetyl monoxime method), serum urea, weight of kidney.

Creatinine clearance : Urinary creatinine X Urinary volume / Serum creatinine

Lipid peroxidation in kidney[7]: Evaluated as malondialdehyde method (MDA)[8]. The animals were sacrificed by decapitation on day 22. The kidneys were dissected out, weighed, homogenized in 1.5% KCl. To 1 ml of Homogenate, 2.5 ml of trichloroacetic acid (TCA, 20%) were added and centrifuged. The supernatent liquid were dissolved in 2.5 ml of 0.05M sulphuric acid and 3.5 ml of thiobarbituric acid, incubated at 37^{o}C for 30 minutes. Contents were extracted into n-Butanol and spectrophotometrically absorbance were observed at 530 nm.

Antioxidant studies-nitric oxide scavenging activity[9,10]: Sodium nitroprusside (5mM) were mixed with MZL, ethanolic extract of PaSE, PuSE and chloroform extract of *Zingiber officinale* Rosc, the solution mixture were incubated at 25^{o}C for 5 hrs. Griess reagent (2% orthophosphoric acid), 1g of sulphanilamide, 100mg of N-napthylethylene diamine were added, made upto 100 ml with distilled water and absorbance measured at 546nm.

Histopathological studies[11-12]: Two animals from each group were sacrificed on day fifteenth and sixteenth and the kidneys were isolated and weighed, sectioned longitudinally and kept in 10% neutral formalin solution. These kidney were embedded in paraffin wax and sections were stained with hematoxylin and eosin and observed under microscope.

Statistical analysis: Statistical analysis were carried out by one way analysis variation (ANOVA) followed by Dunnet's test. . *p< 0.05, **p< 0.01, ns - non significant. The values are expressed as mean ± SEM.

Results and Discussion:

LPO in kidney tissue increases in cisplatin treated mice (groupII) comparing with control (groupI), where as groupIII (extract of PaSE used) and groupIV (extract of PuSE used) showed significant decrease of LPO. Nitric oxide scavenging activity showed less in case of cisplatin treated mice with comparing to control, as well as groupIII (extract of PaSE used) and groupIV (extract of PuSE used), where it showed significant antioxidant property(Table1).

Histopathological features of the kidney like glomerular congestion, blood vessel congestion, intestinal edema, inflammatory cells, necrosis, tubular casts were observed and investigation showed that, in groupI control there are no abnormalities in these parameters and whereas there are very high abnormalities in groupII cisplatin treated mice. In case of groupIII (extract of PaSE used) and groupIV (extract of PuSE used) showed significant reduction of these abnormalities with comparing to cisplatin treated mice (Table2).

It is found that, the ethanolic PaSE & PuSE extract treatment on mice showed significant increase in body weight (%), creatinine clearance and significant decrease in urinary protein **(**UP), serum total protein (S_{TP}), urine to serum creatine ratio(Ucr/Scr), creatinine clearance **(**SC), serum uric acid (SUA), blood urea nitrogen (BUN), serum urea (SU), weight of kidney (g) as compared to cisplatin treated mice, indicating nephroprotectivity (Table3).

Histopathological studies: In histopathological slides of control mice kidney showed normal tubular epithelial cells and glomeruli, whereas cisplatin treated mice showing glomerular atrophy, infiltration of cells, tubular congestion. The treatment of ethanolic extract of PaSE (groupIII) & PuSE (groupIV) showed regenerative changes in glomeruli and tubules, normalicy regaining of tubular epithelial cells, as well as in renal corpuscles/parietal epithelial (Figure1).

Table 1: LPO kidney tissue and Nitric oxide scavenging activity in cisplatin-induced nephrotoxicity mice.

Groups	LPO in kidney tissue	Nitric oxide scavenging activity
I	5.830 ± 0.384	20.630 ± 0.518
II	11.04 ± 0.671	10.25 ± 1.955**
III	6.723 ± 0.477	16.90 ± 2.670
IV	6.723 ± 0.485	14.440±5.354**
V	9.152 ± 1.038	16.770 ± 2.017
VI	8.445± 2.414	13.89 ± 2.906**

Table 2: Histopathological features of the kidney.

Groups	Glomerular congestion	Blood vessel congestion	Intestinal edema	Inflammatory cells	necrosis	Tubular casts
I	--	--	--	--	--	--
II	+++	++++	+++	+++	++	+++
III	++	+	-	+	--	--
IV	+	-	+	-	+	+
V	-	+	-	+	-	--
VI	-	-	+	-	+	+

++++ = very high, +++ = High, ++ = medium, + = low, - = negative.

Table 3: Parameters studied for nephroprotective activity of the effect of alcoholic extract on cisplatin-induced nephrotoxicity on gross behavioural studies.

Groups	creatinine clearance (ml/hr/100g bd.wt)	Urinary protein (mg/24h	S_{TP} (g/dl)	Ucr/Scr, (data4)	Body weight change (%)	Serum creatinine (mg/ml)	Serum uric acid (mg/ml)	Blood urea nitrogen, BUN (mg/ml)	Serum urea (mg/ml)	Weight of kidney (g)
I	8.877 ±0.565	3.470 ±0.388	2.613 ±0.289	9.505 ±0.417	5.047 ±0.221	0.690 ±0.049	1.730 ±0.034	8.33 2 ±0.674	17.581 ±1.039	0.313 ±0.029
II	4.620 ±0.457**	6.533 ±0.361**	4.285 ±0.388**	7.147 ±0.472**	2.742 ±0.188**	1.345 ±0.039**	3.190 ±0.104**	21.103 ±0.559**	31.540 ±0.509**	0.523 ±0.107*
III	6.855 ±0.554**	4.535 ±0.298**	3.983 ±0.330**	6.455 ±0.316**	3.238 ±0.288**	0.796 ±0.061	1.930 ±0.032	11.330 ±0.546	28.170 ±1.384	0.510 ±0.046*
IV	5.380 ±0.520**	5.450 ±0.370**	3.553 ±0.379**	6.152 ±0.286**	4.185 ±0.657*	1.808 ±0.107	1.880 ±0.044**	9.982 ± 0.882	18.930 ±0.677	0.520 ±0.131*
V	7.237 ±0.418**	6.600 ±0.398**	4.258 ±0.413**	6.440 ±0.329**	3.750 ±0.657**	0.963 ±0.062**	1.900 ±0.028	14.772 ±4.782**	26.840 ±1.640**	0.715 ±0.064**
VI	5.287 ±0.430**	4.722 ±0.465**	3.892 ±0.373**	8.620 ±0.525**	3.532 ±0.594**	0.763 ± 0.076	1.830 ±0.056	14.210 ±1.402**	23.000 ±1.728**	0.555 ±0.124**

Ucr/Scr = urine to serum creatine ratio. The values are expressed as mean ± SEM of 6 animals. Statistical significance test for comparison were done by ANOVA, followed by Dunnett's test. , *p< 0.05, **p< 0.01.

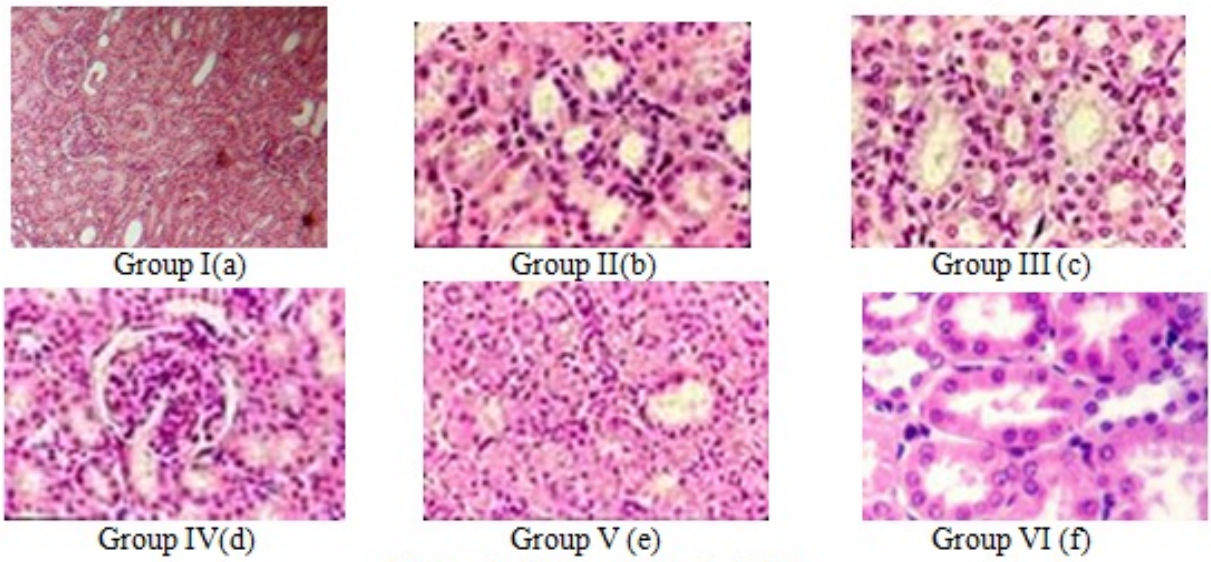

Figure 1: Histopathological slides

GroupI(a): Normal mice kidney tubular epithelial cells and glomeruli by a section through kidney.

GroupII(b): Treated with cisplatin showed glomerular atrophy, infiltration of cells, tubular congestion.

GroupIII(c): Treated with cisplatin and extract of PaSE Showed regenerative changes in glomeruli and tubules.

GroupIV(d): Treated with cisplatin and extract of PuSE showed regenerative changes in glomeruli and tubules.

GroupV(e): Treated with cisplatin and ZE showing normalicy regaining of tubular epithelial cells and glomeruli.

GroupVI(f): Treated with cisplatin and MZL showing renal corpuscles, parietal epithelial normalicy regaining by a section through kidney.

Conclusion:

From the above study it is concluded that Nephroprotective evaluation of ethanolic extract of PaSE and PuSE showed their nephroprotectivity. Histopathological study showing normalicy regaining of tubular epithelial cells, glomeruli and also mainly reduction of serum total protein, Ucr/Scr, Body weight change(%), serum creatinine, seru uric acid, blood urea nitrogen, serum urea, weight of kidney and increase of creatinine clearance, urine protein after our extract administration, upto the extent proofs having nephroprotective activity.

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