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Full Length Research Paper

EFFECT OF FRUITS OF *PEDILUM MUREX* AGAINST CADMIUM CHLORIDE-INDUCED NEPHROTOXICITY IN RATS

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ABSTRACT

The purpose of present study is to evaluate the nephroprotector activity of the ethanolic and aqueous extracts of fruits of Pedalium murex (300 and 600mg/kg body weight, p.o.) against cadmium chloride-induced (3mg/kg/d s.c.) renal toxicity in rats. The effect of plant extracts were examined in terms of blood urea nitrogen, serum creatinine, urinary protein, urine to serum creatinine ratio, lipid peroxidation, gluthione,catalase in kidney.In present study, Cadmium induced nephrotoxicity characterized by significant elevation of serum markers levels, increased urinary protein excretion, raised LPO levels, reduced GSH and CAT levels, reduced creatinine clearance. Co-administration of either ethanolic or aqueous extract with $CdCl_2$ was significantly prevented the renal injury in dose dependent manner. The present study provides the corroborative scientific evidence for the folklore use of Pedalium murex in urinary troubles.

***Key Words**: Cadmium chloride, Pedalium murex , Nephrotoxicity.*

INTRODUCTION

Cadmium is a well known environmental toxin. The toxicity of cadmium as an industrial pollutant, a food contaminant and as one of the major components in cigarette smoke has been well established. The kidney and the liver are the major target organs of accumulation and intoxication. Chronic exposure to cadmium can damage the renal proximal tubular epithelial cells of S_1 and S_2 segments [1]. This damage can cause proximal tubular dysfunction in both humans and experimental animals [2] manifested by proteinuria glucosuria, aminoacid uria and phosphaturia [3]. various reports suggested that several plants and compounds possess antioxidant properties are exhibited nephroprotector activity against cadmium chloride induced renal damage. *P*edalium murex (pedaliaceae; *P. murex)*is one such plant containing triterphenoids, fatty acids, steroids and flavonoids and also used to treat rheumatism, urinary calculi and renal troubles by village folk of Rayalaseema [4, 5] and absence of experimental data to justify the nephroprotective activity of fruits of *P. murex.* Hence, the present study was designed the systematic pharmacological evaluation of fruits of *P. murex* against experimentally induced renal damage by cadmium chloride.

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MATERIALS AND METHODS:

Plant material: Fruits of *Pedalium murex* were collected from Talakona, chiittor district, Andhra Pradesh, India in the month of August- December, 2007 and authenticated by botanist Dr. Madhava chetty, Herbarium keeper, Department of botany, Sri venkateswara university, Tirupati, India and specimen (Specimen No:862) has been deposited in Department of botany, Sri venkateswara university, Tirupati, India.

Preparation of Plant extracts;

***Pedalium murex* Ethanol Extract (PEE):** The fruits were allowed to dry under shade. The dried fruits (500 g) were powdered in a Wiley mill and extracted with rectified spirit (4L x3). The extract was concentrated under reduced pressure to get solid mass 40g (8%).

***Pedalium murex* Aqueous Extract (PAE):** Fruit powder (400g) was boiled with water (2.5L) for 30 min, cooled, kept over night at room temp (25± 2^{o}C) and filtered. The filtrate was concentrated (100 mg/ml) and was used for the present study.

Animals: Healthy wistar adult male albino rats between 2 and 3 months of age and weighing about 150-200g were used to the study. Housed in polypropylene cages and fed with standard rat pellet diet, water *ad libitum.* Animals were acclimatized to our lab environment for about a week. The study was conducted after obtaining Institutional ethical committee clearance.

Treatment Protocol:

Effect of PEE and PAE in normal rat kidney:

The animals were divided into three groups of six rats each randomly.

Animals of group I (control) received 2% gum acacia in distilled water (10 ml/kg/d) for 8 days. Animals of group II_{PEE} received PEE (600 mg/kg) suspended in the vehicle (10 ml/kg) for 8 days .Animals of group III_{PAE} received PAE (600 mg/kg) suspended in the vehicle (10 ml/kg) for 8 days.

Effect of PEE and PAE On cadmium chloride induced kidney damage in rats:

The rats were divided into seven groups of six animals each.

Animals of Group I (Normal control) received 2% gum acacia (10 ml/kg/d) for 8 days. Animals of group II received 2% gum acacia and water (10 mg/kg/d) for 8 days. Animals of group III received PEE (300 mg/kg) suspended in the vehicle (10 ml/kg) for 8 days. Animals of group IV received PEE (600 mg/kg)) suspended in the vehicle (10 ml/kg) for 8 days. Animals of group V received PAE (300 mg/kg)) suspended in the vehicle (10 ml/kg) for 8 days .Animals of group VI received PAE (600 mg/kg)) suspended in the vehicle (10 ml/kg) for 8 days. Animals of group VII received Vitamin E (100 mg/kg)) suspended in the vehicle (10 ml/kg) for 8 days.

In addition to this, the animals in groups II, III, IV, V, VI and VII were co-administered with cadmium chloride [6] from day four to day eight. Cadmium chloride (3 mg/kg/d) was injected subcutaneously in neck region in a volume of 1 ml/kg. Group I received normal saline instead of cadmium chloride ($CdCl_2$).

On the day 9, urine was collected with the help of metabolic cages and the urine samples were subjected for estimation of urinary functional parameters. The animals were sacrificed by cervical decapitation and blood samples were collected by cardiac puncture and were used for estimation of serum markers.

Nephroprotector Activity was assessed by estimating Blood Urea Nitrogen (BUN) by DAM method [6], Serum Creatinine (SC) by Jaffe.s Alkaline Picrate method [6], Urinary Total Proteins (UTp) by Turbidimetry method, Urinary Creatinine (Ucr) byAlkaline picrate Method[6],, Creatinine Clearence (Clcr) Clcr was calculated by using formula

Creatinine clearance = Urinary creatinine X urinary volume/hr/ Serum creatinine.

Lipid Peroxidation (LPO), Glutathione (GSH), Catalase activities (CAT) in kidney tissue was estimated by following standard methods [7].

Statistical Analysis: The statistical data was presented as mean ±SEM, Parametric data which include all the biochemical parameters were analyzed using a paired t' test for the paired data or one way analysis of variance (ANOVA) followed

by a Dun net multiple comparisons post test. A probability value of P<0.05 was considered as significant.

RESULTS:

Effect of *P. murex* fruit extracts on normal rat kidney: Animals which received the PEE (group II_{PEE}) and animals which received the PAE (III_{PAE}) alone for eight days exhibited no change in serum markers level, urinary functional parameters and antioxidant enzyme levels. Hence, the alcoholic extract and aqueous extracts did not show any deteriorative effects on kidney (Table-1).

Table-2 lists the effect of PEE, PAE of *P.murex* on cadmium-induced nephrotoxicity. Administration of cadmium at 3 mg / kg s.c. caused significant elevation of BUN, SC and increased urinary protein in group II animals, when compared to normal control animals (group I). On co-administration of PEE (group III and IV animals) and PAE (group V and VI animals) of plant with $CdCl_2$, a significant dose Dependent reduction in the levels of BUN, SC and urinary protein extraction was observed when compared to group II animals.

Table-1: Effect of ethanolic and aqueous extracts of *p.murex* on Normal rats

Group	Treatment (mg/kg)	BUN (mg/dl)	SC (mg/dl)	U_{TP} (mg/24hrs)	Clcr (ml/hr/100g bd.wt)	LPO (nmol min^1 mg $tissue^{-1}$)	GSH (µmol min^1 mg $tissue^1$)	CAT (K/ min)
I.	Normal	23.28±0.16	0.66±0.14	7.20±0.33	18.0±1.5	64.58±2.13	12.14±0.13	1.64±0.12
II_{PEE}	PEE (600mg/kg)	24.24±0.19#	0.67±0.01#	7.26±0.19#	18.8±1.7#	66.28±3.07#	12.56±0.10#	1.53±0.09#
III_{PAE}	PAE (600mg/kg)	23.90±0.13#	0.66±0.15#	7.28±0.16#	18.3±1.9#	65.25±3.10#	12.44±0.17#	1.66±0.15#

Each value represents the mean ± S.E.M from 6 animals in each group;.# NS When compared with Normal.; PEE –*P.murex* ehanolic extract; PAE–*P.murex* aqueous extract.

Table-2: Effect of ethanolic and aqueous extracts of fruits of *p. murex* on $CdCl_2$-induced nephrotoxicity

Group	**Treatment (mg/kg)**	**BUN (mg/dl)**	**SC (mg/dl)**	**U_{TP} (mg/24hrs)**	**Clcr (ml/hr/100g bd.wt)**
I.	Normal	23.4 ± 1.16	0.67±0.11	7.25±0.37	18.00±1.50
II.	$CdCl_2$ (3 mg/kg)	37.26 ± 2.13*	1.87±0.12*	16.36±0.24*	7.10±0.57*
III.	PEE (300)+$CdCl_2$	32.44±1.24 ab	1.62±0.12ab	13.25 ± 0.24ab	10.50±1.30ab
IV.	PEE (600)+$CdCl_2$	28.90±2.17ab	0.81±0.17ab	10.48 ± 0.16ab	15.00±0.94ab
V.	PAE (300)+$CdCl_2$	29.70±2.15ab	1.14±0.15ab	11.14 ± 0.21ab	12.00±1.70ab
VI.	PAE (600)+$CdCl_2$	24.18±1.28ab	0.79±0.16ab	8.60 ± 0.30ab	17.50±1.50ab
VII.	Vit.E (100 mg/kg) + $CdCl_2$	23.98±1.25a	0.70±0.17a	8.01 ± 0.35a	17.70±1.70a

Each value represents the mean ± S.E.M from 6 animals in each group
*P<0.05 when compared with normal control group. PEE –*P.murex* ehanolic extract
a: P<0.05 when compared with control group($CdCl_2$) PAE–*P.murex* aqueous extract
b : P<0.01 when compared with Std group.

Effect on *in vivo* antioxidant activity: Animals which received $CdCl_2$ alone increased levels of LPO (97.26±1.17) decreased the GSH (7.4±0.28) and CAT (0.96±0.26) levels when compared to normal control animals (64.62±1.15, 12.19±0.23, 1.64±0.05).

Co-administration of ethanolic extract (Gr-III & IV) or aqueous extract (Gr-V & Gr-VI) exhibited decrease in LPO levels (Gr-III 83.16±1.06, Gr-IV

80.50±2.21, Gr-V 76.00±2.14 and Gr-VI 67.04±2.14) (Fig-1), significant increase in GSH (Gr-III 10.60±0.37, Gr-IV 11.36±0.20, Gr-V 111.66±0.33 and Gr-VI 12.00±0.40) (Fig-2) and CAT levels (Gr-III 1.32±0.09, Gr-IV 1.48±0.12, Gr-V 1.59±0.25 and Gr-VI 1.72±0.32) (Fig-3). Administration of $CdCl_2$ in Gr-II animals showed significant decrease in levels of GSH and CAT when compared to Gr-I animals. Animals which received PEE and PAE exhibited dose dependent increase of GSH and CAT levels.

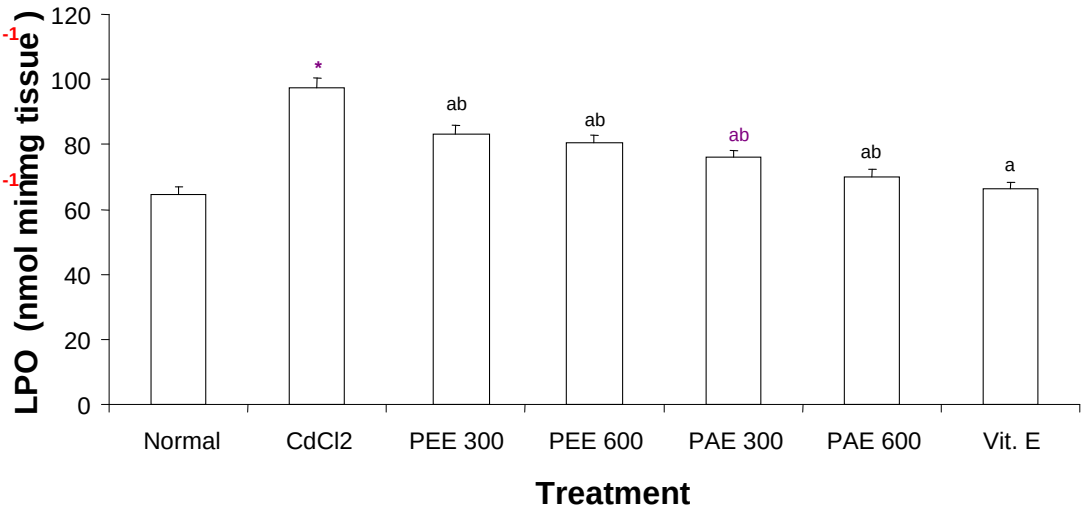

Fig-1: Effect of ethanolic and aqueous extracts of fruits of *P. murex* on LPO in CdCl2 -induced nephrotoxicity
Each value represents the mean ± S.E.M from 6 animals in each group.:
*P<0.05 when compared with normal control group.:
[a]P<0.05 when compared with control group ($CdCl_2$)[b]
P<0.01 when compared with Std group

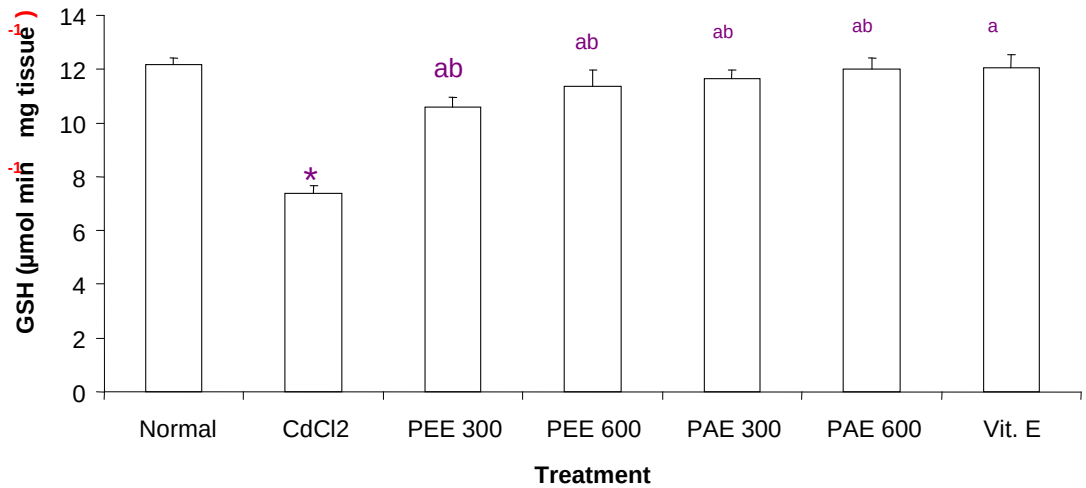

Fig-2: Effect of ethanolic and aqueous extracts of fruits of *P. murex* on GSH in CdCl2 -induced nephrotoxicity
Each value represents the mean ± S.E.M from 6 animals in each group.

*P<0.05 when compared with normal control group
[a]P<0.05 when compared with control group ($CdCl_2$)
[b]P<0.01 when compared with Std group

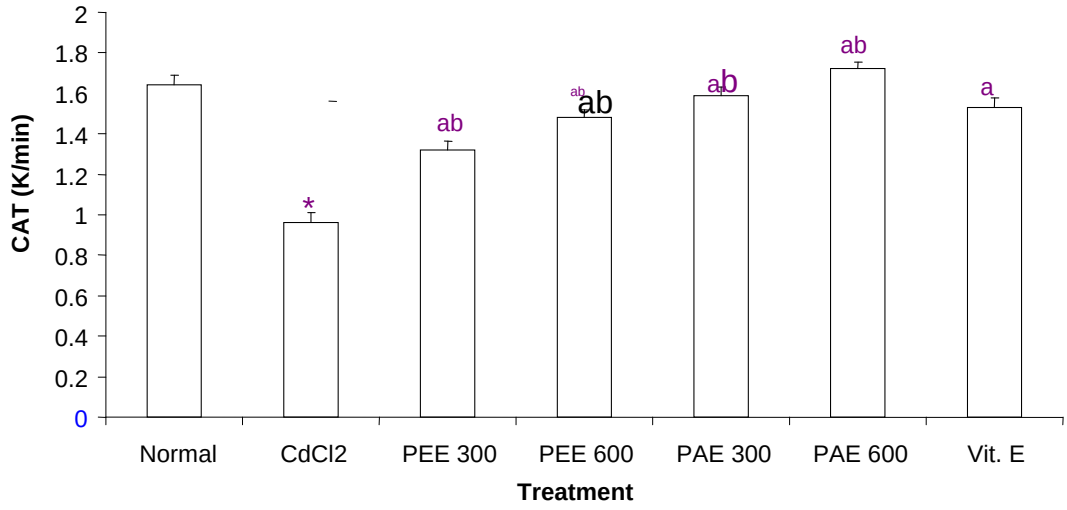

Fig-3: Effect of ethanolic and aqueous extracts of fruits of *P. murex* on CAT in CdCl2 induced nephrotoxicity
Each value represents the mean ± S.E.M from 6 animals in each group.
*P<0.05 when compared with normal control group:
[a] P<0.05 when compared with control group ($CdCl_2$):
[b]P<0.01 when compared with Std group

DISCUSSION

Cadmium (Cd) is an environmental pollutant causing human health problems, with the kidney being a primary target organ. In across sectional population study environmental Cd exposure was shown to associated with renal dysfunction [8]. Biological half of cd is more than 30 years hence nearly all the Cd ingested is retained [9]. The primary nephrotoxicity associated with the exposure of this Cd involves the renal proximal tubule[2].Renal injury is believed to be caused by Cd-metallothionein that is originally produced in liver, released into circulation taken up by the renal proximal tubular epithelial cells and degraded to liberate toxic Cd ions [10,11] . The biochemical mechanism of Cd-induced nephrotoxicity appears to involve oxidative stress [12]. Previous reports evidenced that the treatment with antioxidants such as Vit.E, N-acetyl cysteine [13], selenium [14] and plants like *Scoparia dulcis* [15], *Rheum emodi* [8], *Aswagandha* [16], Grape seeds [17] showed protection against $CdCl_2$ -induced toxicity.

Search for potent nephroprotective agent has made man turn to suitable sources *viz.* indigenous system of medicine with less side effects. It is well documented fact that most of the medicinal plants are enriched with bio-flavonoids which have antioxidant property. *Pedalium murex* is one such plant contain number of bioactive compounds and it's fruits used to treat kidney disorders in folklore medicine [5]

Hence, the present study was focused on the effect of ethanolic and aqueous extracts of *P. murex* fruit on the renal damage induced by $CdCl_2$. Nephroprotector activity was assessed by (1) determination of serum marker levels and urinary functional parameters, (2) determination of antioxidant enzyme activities such as alone for 8 days, there was no change in serum markers levels, urinary functional parameter levels and antioxidant

enzyme levels are same to that of normal animals. Hence ethanolic and aqueous extracts of *P. murex* did not show any deteriorative effect on kidney.

$CdCl_2$ caused renal failure characterized by elevation of serum marker levels, deteriorated the renal functional parameters indicated by increased the urinary protein excretion and decreased the Clcr which is also evidenced by earlier reports. Protective effects of ethanolic and aqueous extracts of *P.murex* was tested at two doses *i.e*, 300 and 600 mg/kg/*p.o* against $CdCl_2$-induced nephrotoxicity. Both extracts (PEE and PAE) were tested at 600 mg/kg against Cd-induced toxicity. The protective effects of PEE and PAE were compared with vitamin E (100 mg/kg) as standard drug.

The success of *P. murex* extracts in reducing SC, BUN, urinary protein excretion and raise in Clcr could be attributed due to its antioxidant properties because of its antioxidant property it reduces the formation of ROS which may be involved in the impairment of GFR. $CdCl_2$ alone significantly increase in LPO levels while GSH and CAT activities were reduced in the kidney tissue similar to earlier studies [15,18]. Co-administration of PEE and PAE (600 mg/kg) extracts significantly decreased LPO, increased the levels of GSH and CAT and as similar to that standard drug treated animals [11, 19].

Previous reports on phytochemical studies suggested that fruits of *P. murex* contain a number of flavanoids like Pedalitin, dinatin [20] luteolin [21] leaves contain flavonoids, quarcetin, dinatin [22].

Flavonoids exhibit several biological activities including antioxidant and free radical scavenging abilities [23]. A relationship between oxidative stress and renal toxicity has been well documented in many experimental animal models. Hence the presence of these constituents in *P. murex* may be responsible for the nephroprotector activity.

CONCLUSION:

In conclusion, the fruits *Pedalium murex* exhibited good nephroprotective effect against $CdCl_2$ induced nephrotoxicity may be through antioxidant property. However further studies are essential to elucidate the exact mechanism of nephroprotector activity.

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