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Research Article

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Influence of aqueous and diethyl ether extracts of *Pongamia pinnata* flowers on lead acetate induced nephrotoxicity

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ABSTRACT

In the present study the nephroprotective potential of aqueous and diethyl ether extract of Pongamia pinnata flowers were assessed in lead acetate induced nephrotoxic rats. The oral administration of water soluble fraction of the diethyl ether extract at a dose of 150 mg/kg b.wt/ day for 90 days exhibited a significant (P < 0.05) reduction in the levels of urea, uricacid and creatinine compared to the aqueous extract treated rats. The renal marker of kidney toxicity such as urea, uricacid and creatinine and antioxidants like enzymatic and non-enzymatic were changed significantly in lead acetate treated rats. Treatment with water soluble fraction of diethyl ether extract and aqueous extract of Pongamia pinnata flowers restored the above altered parameters significantly in lead acetate inducted animals. The water soluble fraction of the diethyl ether extract showed a more efficient antinephrotoxic effect compared to the aqueous extract.

Key words: *Pongamia pinnata*, Lead acetate, Nephrotoxicity, Antioxidants, Carvedilol.

INTRODUCTION

The kidney is often a target for the toxic substances to which man is exposed. An impression of the frequency with which renal insufficiency is caused by toxic substances[1]. Drug induced nephrotoxicity is one of the top priority in the world. About one third of the cases of nephrotoxicity is united states are due to drug [2]. Nephrotoxicity (from Greek: nephors, "kidney") is a poisonous effect of some substances, both toxic chemicals and medication, on the kidneys. There are various forms of toxicity [3], for example Chronic accumulation of lead in the body eventually leads to impairment in renal function[4].

A review of literature indicated that *Pongamia pinnata* has many medicinal uses; anti-diabetic activity [5]. All parts of the plant have been used as a crude drug for the treatment of tumours, piles, skin diseases, itches, abscess, painful rheumatic joints wounds, ulcers, diarrhoea etc.,[6,7]. The effectiveness of *P. pinnata* as a source of biomedicines has been reported[8] specifically as antimicrobial and therapeutic agents. This paper examines the efficacy of ehyl acetate extract of *Pongamia pinnata* as a hepato protective agent.

The activity of renal markers such as urea, uricacid, creatinine and the endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione S-transferase (GST) and reduced glutathione (GSH), vitamin C and vitamin E were measured from kidneys homogenates. Further, the level of lipid peroxidation were also estimated from renal samples.

EXPERIMENTAL SECTION

Experimental Animals

Healthy adult male albino Wistar rats and the weight of the animals ranged (160-180 g) were selected and housed in polypropylene cages layered with husk and kept in a semi-natural light/dark condition (12 h light/12 hours dark). The animals were allowed free access to water and standard pellet diet (Amrut Laboratory Animal Feed, Pranav Agro Industries Ltd., Bangalore, India). The animals were cared in accordance with the "Guide for the care and use of laboratory animals" (NIH, 1985) and "Committee for the purpose of Control and Supervision on Experimental Animals" (CPCSEA, 2004).

Source of chemical

All other chemicals and solvents were of analytical grade and purchased from S.D. Fine Chemicals, Mumbai and Himedia Laborateries Pvt. Ltd., Mumbai, India.

Experimental induction of renal toxicity

Renal toxicity was induced by the oral administration of freshly prepared of lead acetate solution (160 mg/kg b.wt./day) [9].

Preparation of the *Pongamia pinnata* extract

The flowers were collected and dried in shade for 15 days and made to coarse powder. The powder was passed through sieve No.40 to achieve uniform particle size and then used for extraction process. A weighed quantity of the powder was subjected to continuous hot extraction in soxlet apparatus with diethyl ether and water, The extract was evaporated under reduced pressure using rotovac evaporator until all solvent was removed to give a molten extract. Those extract of *P.pinnata* was used for the study.

Experimental design

Rats were divided into the following groups.

Group 1: Control rats.
Group 2: Rats continued to receive lead acetate and considered as toxic control.
Group 3: Rats were administered carvedilol (5 mg/kg b.wt/ day with 0.5 % methyl cellulose to facilitate dissolution and absorption) along with lead acetate.
Group 4: Rats were administered diethyl ether extracts of *Pongamia pinnata* (150 mg/kg b.wt./ day) along with lead acetate.
Group 5: Rats were administered aqueous extracts of *Pongamia pinnata* (150 mg/kg b.wt./ day) along with lead acetate.

After 90 days of treatment, the animals were fasted for 12 h and sacrificed by cervical dislocation. Blood was collected in a cleaned tube with a mixture of potassium oxalate and sodium fluoride (1:3) for the estimation of various biochemical parameters.

Assay of biochemical parameters

The level of serum urea was estimated by using the diagnostic kit based on the method of Fawcett and Scott (1960) [10], serum creatinine by the method (Tietz, 1987)[11], using colour reaction (Jaffe's, 1886) [12], serum uric acid by the enzymic method described by Caraway (1955) [13]. Lipid peroxidation in tissues by the method of Ohkawa *et al.* (1979) [14] , CD in the tissues by the method of Recknagel and Glende (1984) [15], Tissue LOOH by the method of Jiang *et al.*, (1992) [16]. SOD was assayed by the method of Kakkar *et al.*, (1984) [17] and the activity of CAT by the method of Sinha, (1972)[18]. The activity of GPx by the method of Rotruck *et al.*,(1973)[19].Glutathione reductase was assayed according to the method of Carlberg and Mannervik (1975)[20]. Glutathione S-transferase (EC 2.5.1.18; GST) activity was assayed according to the method of Habig *et al.*, (1974) [21] and GSH by the method of Boyne and Ellman (1972) [22]. Vitamin C was measured according to the method of Roe and Kuether., (1943) [23] , and Vitamin E in tissues by the method of Desai (1971) [24].

Statistical Analysis

All quantitative measurements were expressed as means ± SD for control and experimental animals. The data were analyzed using one way analysis of variance (ANOVA) with the help of SPSS/PC (statistical package for social

sciences, personal computer) and the group means were compared by Duncan's Multiple Range Test (DMRT). The results were considered statistically significant if the p value is less than 0.05.

RESULTS

The effect of diehyl ether and aqueous extract of *Pongamia pinnata* on renal function markers levels are shown in table 1. The levels of urea, uric acid and creatinine significantly increased in lead acetate induced rats and treatment with diehyl ether and aqueous extract of *Pongamia pinnata* and carvedilol significantly decreased the levels of urea, uric acid and creatinine.

As depicted in tables 2, lipid peroxidation (measured by the levels of TBARS, LOOH and CD) increased in animals administered with lead acetate. The extent of lipid peroxidation was maintained at normal levels in rats coadministered with diehyl ether and aqueous extract of *Pongamia pinnata.* In all the parameters studied, the effect of diehyl ether and aqueous extract of *Pongamia pinnata* was comparable to that of carvedilol.

Tables 3 show the activities of SOD, CAT, GPx, GR and GST in the tissues of control and experimental animals. The activities of these antioxidant enzymes were remarkably decreased in tissues of lead acetate-administered group. In response to diethyl ether and aqueous extract of *Pongamia pinnata* treatment, the activities of these enzymic antioxidants increased. The effect of diethyl ether and aqueous extract of *Pongamia pinnata* was as effective as carvedilol.

The levels of non-enzymic antioxidants such as reduced glutathione, ascorbic acid and α-tocopherol (table 4) were found to be decreased significantly in the kidney tissues studied in leadacetate-administered rats. Coadministration of diethyl ether and aqueous extract of *Pongamia pinnata* raised these antioxidants to near normal levels. There were no significant difference between diethyl ether and aqueous extract of *Pongamia pinnata* and carvedlol coadministered rats.

Table 1. Levels of Serum Urea, creatinine and uric acid in the control and treated rats

Experimental Groups	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
Group I (Control)	15.23±1.46[a]	0.55 ± 0.05[a]	0.80 ± 0.07[a]
Group II (Lead acetate)	24.97 ± 2.40[b]	1.16 ± 0.11[b]	1.59 ± 0.15[b]
Group III (Lead acetate + Carvedilol)	16.52± 1.59[a]	0.52± 0.05[a]	0.85 ± 0.08[a]
Group IV (Lead acetate + Diethyl acetate extract of *Pongamia pinnata*)	18.29 ± 1.76[a]	0.79 ± 0.07[a]	1.28 ± 0.12[a]
Group V (Lead acetate + Aqueous extract of *Pongamia pinnata*)	20.81 ± 1.95[a]	0.97 ± 0.09[a]	1.34 ± 0.13[a]

Values are expressed as mean ±SD for 6 rats in each group. Values not sharing a common superscript letter differ significantly at p < 0.05 (DMRT).

Table 2: Levels of lipid peroxidative markers of kidney in the control and treated rats

Experimental Groups	TBARS (mmol /mg tissue)	LOOH (mmol/mg tissue)	CD (mmol/mg tissue)
Group I (Control)	0.52 ± 0.05[a]	53.67 ± 5.16[a]	11.07 ± 1.06[a]
Group II (Lead acetate)	2.02 ± 0.19[b]	153.42 ± 14.76[b]	22.27 ± 2.14[b]
Group III (Lead acetate + Carvedilol)	0.5 ± 0.04[a]	72.09 ± 6.94[a]	13.81 ± 1.32[a]
Group IV (Lead acetate + Diethyl acetate extract of *Pongamia pinnata*)	0.90 ± 0.08[a]	86.35 ± 8.31[a]	18.23 ± 1.75[a]
Group V (Lead acetate + Aqueous extract of *Pongamia pinnata*)	1.23 ± 0.11[a]	93.03 ± 8.95[a]	19.70 ± 1.89[a]

Values are expressed as mean ±SD for 6 rats in each group. Values not sharing a common superscript letter differ significantly at p < 0.05 (DMRT)

Table 3. Changes in the Enzymatic antioxidant activities of SOD, CAT, GPx, GR and GST of control and treated rats

Groups	SOD	CAT	GPx	GR	GST
Group I (Control)	1.77 ± 0.17^a	29.19 ± 2.81^a	7.58 ± 0.73^a	20.31 ± 1.95^a	5.26 ± 0.50^a
Group II (Lead acetate)	0.97 ± 0.09^b	21.13 ± 2.03^b	5.26 ± 0.50^b	13.19 ± 1.27^b	2.57 ± 0.24^b
Group III (Lead acetate + Carvedilol)	1.71 ± 0.16^a	25.94 ± 2.49^a	7.40 ± 0.71^a	19.09 ± 1.83^a	5.14 ± 0.49^a
Group IV (Lead acetate + Diethyl acetate extract of *Pongamia pinnata*)	1.37 ± 0.13^a	25.02 ± 2.40^a	5.22 ± 0.50^a	15.22 ± 1.46^a	3.01 ± 0.29^a
Group V (Lead acetate + Aqueous extract of *Pongamia pinnata*)	1.10 ± 0.10^a	20.52 ± 1.97^a	5.09 ± 0.49^a	15.42 ± 1.48^a	2.94 ± 0.28^a

Values are expressed as mean ±SD for 6 rats in each group. Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT).

SOD: Enzyme required for 50% inhibition of NBT reduction/min/mg Hb

CAT: µM of H_2O_2 utilized/min/mg Hb.

GR: µmoles of NADPH oxidized/min/mg Hb

GST: µmoles of CDNB-GSH conjugate formed/min/mg Hb

Table 4. Changes in the Non-enzymatic antioxidant activities of GSH, Vitamin-C and Vitamin-E of control and treated rats

Groups	GSH	Vitamin C	Vitamin E
Group I (Control)	10.77 ± 1.03^a	0.42 ± 0.04^a	2.40 ± 0.23^a
Group II (Lead acetate)	8.09 ± 0.78^b	0.14 ± 0.01^b	0.89 ± 0.08^b
Group III (Lead acetate + Carvedilol)	10.46 ± 1.00^a	0.36 ± 0.03^a	2.38 ± 0.23^a
Group IV (Lead acetate +Diethyl acetate extract of *Pongamia pinnata*)	8.15 ± 0.78^a	0.21 ± 0.02^a	0.98 ± 0.09^a
Group V (Lead acetate +Aqueous extract of *Pongamia pinnata*)	8.06 ± 0.77^a	0.13 ± 0.01^a	1.03 ± 0.09^a

Values are expressed as mean ±SD for 6 rats in each group. Values not sharing a common superscript letter differ significantly at $p < 0.05$ (DMRT).

GSH: mg/dL, Vit-C & Vit-E : mg/dL

DISCUSSION

Urea is the major nitrogen containing metabolic product of protein metabolism; uric acid is the major product of purine nucleotides; creatinine is endogenously produced and released into body fluids and its clearance measured as an indicator of glomerular filtration rate[25,26]. The results from the present study showed that lead acetate-rats significantly increased the levels of urea, uric acid and creatinine in serum which are considered as significant markers of renal dysfunction. The levels of these renal function markers were much lower in case of lead acetate nephrotoxic rats treated with diethyl ether and aqueous extract of *Pongamia pinnata*. It shows that diethyl ether and aqueous extract of *Pongamia pinnata*, to an extent, preserves the functional capacity of the kidney from the adverse effects of lead acetate.

Excessive LPO as measured by the formation of TBARS and/or lipid hydroperoxides and conjugated dienes in ethanol treated rats has been reported by many researchers[27]. In agreement with these findings, lead acetate-administered rats in our present study showed increased levels of TBARS, CD and hydroperoxides in the circulation as compared to control rats. Administration of diethyl ether and aqueous extract of *Pongamia pinnata* to lead acetate- administered rats significantly decreased the levels of these LPO markers as compared to lead acetate alone administered animals. The decrease in LPO on diethyl ether and aqueous extract of *Pongamia pinnata* treatment can be correlated with the elevated levels of antioxidants. The ability of diethyl ether and aqueous extract of *Pongamia pinnata* to enhance the levels of antioxidants along with its antilipid-peroxidative activity suggest that this compound might be potentially useful in counteracting free radical-mediated injuries involved in the development of liver damage caused by heavy metal toxicity.

SOD is an ubiquitous chain breaking antioxidant found in all aerobic organisms. It is a metalloprotein widely distributed in all cells and plays an important protective role against reactive oxygen species induced oxidative damage. SOD converts superoxide ion ($O_2^{\bullet-}$) to hydrogen peroxide (H_2O_2) and the H_2O_2 thus formed is degraded by CAT and GPx. CAT is present in all major body organs of animals and human beings and is especially concentrated in the liver and erythrocytes. Superoxide ion ($O_2^{\bullet-}$) and hydroxyl radical ($OH^{\bullet}$) are known to cause marked injuries to the surrounding tissues and organs[28,29]. The decrease in the SOD and CAT activities may be associated with the elevation of the intracellular concentrations of H_2O_2 [30]. CAT has been reported to be responsible for the detoxification of H_2O_2, which is an effective inhibitor of SOD[31].

The decrease in the SOD activity could be due to the oxidative inactivation of the enzyme due to the excessive reactive oxygen species generation [32] or generation of the α-hydroxyl ethyl radical from ethanol that inactivates SOD [33]. Diethyl ether and aqueous extract of *Pongamia pinnata* supplementation to the lead acetate treated group elevates the SOD and CAT activities of the kidney emphasizing the antioxidant and nephro protective activities of diethyl ether and aqueous extract of *Pongamia pinnata*.

GSH is a tripeptide (L-γ-glutamylcysteinylglycine), an antioxidant and a powerful nucleophile, critical for cellular protection such as detoxification of ROS, conjugation and excretion of toxic molecules and control of inflammatory cytokine cascade[34] . Depletion of GSH in tissues leads to impairment of the cellular defense against ROS, and may result in peroxidative injury. The levels of GSH were significantly decreased in lead acetate treated rats. Moreover in addition to being a direct free radical scavenger, GSH is known to function as a substrate for GPx and GST. The activities of GPx and GST in this study were lowered on lead acetate treatment which may be attributed to the unavailability of GSH. Administration of diethyl ether and aqueous extract of *Pongamia pinnata* to lead acetate treated rats increased the levels of GSH and the activities of GPx and GST. This may be due to their increased utilization to scavenge the significantly elevated levels of ROS that are formed on lead acetate treatment.GR helps to restore the levels of GSH by reducing the oxidized product of glutathione. The activity of GR was also lower upon alcohol administration as compared to control rats which in turn may inactivate many enzymes containing 'SH' groups and inhibit protein synthesis[35].

Non-enzymic antioxidants can also play a critical role in the defense against oxidative stress. In our study, we observed a significant decrease in the levels of the non enzymic antioxidants vitamins C and E which may be due to the enhanced oxidative stress. Vitamin E can act as a chain breaking antioxidant preventing LPO, and any impairment in the antioxidant defense results in free radical- induced cell injury. Vitamin E terminates LPO by trapping free radicals, thereby getting itself converted to α-tocopheroxyl radicals, while vitamin C may have an important role in the regeneration of α-tocopherol from α-tocopheroxyl radical[36,37].The diethyl ether extract was found to be more potent than the aqueous extract.

In conclusion, the lead acetate induced nephrotoxicity may be related with oxidative damage. Co-administration of diethyl ether and aqueous extract of *Pongamia pinnata* decreased the harmful effects of lead acetate both by inhibiting free-radical formation and by restoration of the antioxidant systems. Further investigations on the mechanism of action of *Pongamia pinnata* are required and may have a considerable impact on future clinical treatments of patients with renal failure.

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