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Research Article

PROTECTIVE EFFICACY OF *TERMINALIA CATAPPA* L. LEAVES AGAINST LEAD INDUCED NEPHROTOXICITY IN EXPERIMENTAL RATS

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ABSTRACT

Terminalia catappa L. belongs to Combretaceae family found in tropical and subtropical regions. In this connection, the protective effect of *Terminalia catappa* leaves against lead induced nephrotoxicity in experimental rats was studied. In group II lead induced nephrotoxicity animals the levels of marker enzymes showed noticeable modulations and there was a significant decrease in transaminases and phosphatases. Oral administration of aqueous extract of leaves of *Terminalia catappa* L leaves (100mg/kg), the levels of transaminases, phosphatases and lysosomal marker enzymes were reverted back to near normal (P<0.05) in group III drug treated animals. It was concluded that aqueous extract of *Terminalia catappa* L leaves have a tremendous protective activity against nephrotoxicity.

Keywords: *Terminalia catappa* L. Nephrotoxicity, Lysosomal enzymes, Lead, Marker enzymes,

INTRODUCTION

Various environmental toxicant and clinically useful drugs, can cause severe organ toxicities through the metabolic activation to highly reactive free radical.[1] Lead poisoning is a medical condition, also known as saturnism, plumbism or painter's colic, caused by increased of lead in blood levels, and may cause irreversible neurological damage, hepatic, renal, cardiovascular and reproductive toxicity.[2] Lead is odourless, colourless and tasteless common environmental toxic metal.[3] It has been widely in metal products, cables, pipelines, paints and pesticides. It can enter the human body through uptake of food, water and air which causes damaging effects on human health. [4] An association between lead poisoning and renal diseases in humans has been recognized for over a century.[5] Lead induced toxicity causes, induced reactive oxygen species[6] which are the initiators of peroxidative damage to the membranes[7, 8] and leads to disruption of proximal tubular architecture, with experimental evidence of disturbances in the function of proximal tubular[9].

Lead induced renal dysfunction includes an increase in the urinary excretion of brush-border enzymes such as alkaline phosphatase (ALP) and$^{\gamma}$ -GT, suggesting that brush-border may be an initial target of heavy metals.[10] A research by Flora *et al* [11] showed that the oral administration of lead acetate significantly enhanced the urinary excretion and a corresponding decline in renal activities of alkaline phosphatase. When tubular injury becomes severe, intracellular enzymes like lactate dehydrogenase (LDH) and aspartate transaminase (AST) increases in serum and tubular reabsorption of solutes and water decreases.[12]

Terminalia catappa is a large tropical tree in the Leadwood tree family, belongs to the Combretaceae. The leaves contain several flavonoids, tannins, saponins and phytosterols. Due to this chemical richness, the leaves are used in different traditional medicines for various purposes. In Taiwan, the fallen leaves are used to treat liver diseases, dysentery and diarrhea.[13] In the present study, the protective activity of *Terminalia catappa* leaves against lead induced nephrotoxicity in experimental rats.

MATERIALS AND METHODS

Animals

Healthy male Wistar albino rats of weighing between 110±20g were used for the present study. They were obtained from the Central Animal House Facility, Dr.ALMPGIBMS, Taramani, University of Madras, Chennai (IAEC No: 06/02/11). The animals were kept in polypropylene cages and received standardized rat pellet and water *ad libitum.* All the procedures were done in compliance with the guidelines issued by the Institutional Animal Ethics Committee.

Chemicals

All Chemical including solvents used were of high purity and of analytical grade marketed by Glaxo Laboratories, Mumbai and Sisco Research Laboratories Pvt. Ltd, Mumbai, India.

Preparation of Plant Extract

Aqueous extract of *Terminalia catappa* L. leaves were collected during the month of January from Chidambaram, Tamilnadu, India. The leaves of the *Terminalia catappa* L. was authenticated by Chief Botanist, Captain Srinivasa Multi Drug Research Institute for Ayurveda and Siddha (CCRAS). A voucher specimen has been deposited at the Department of Pharmacology and Environmental Toxicology, Dr.ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Chennai, Tamilnadu, India. The leaves was dried under shade at room temperature for seven days and then reduced to a coarse powder. This powder was used for the preparation of aqueous extracts. The extract obtained was filtered and concentrated to a viscous consistency at 48ºC (the yield was 12.6 % w/w for aqueous extracts).

Experimental Design

In the present investigation rats were divided into four groups of six animals each. Group I: Rats served as control animals were administered with normal saline (0.9%) 1ml intraperitoneally daily for 6 weeks. Group II: Animals injection with lead acetate at a dose of 8 mg/kg body weight intraperitoneally daily for 6 weeks. Group III: After inducing nephrotoxicity, rats were administered with the aqueous extract of *Terminalia catappa* L. leaves orally at a concentration of 100mg/kg body weight for a period of 28 successive days. Group IV: Animals received only plant extract orally at a concentration of 100mg/kg body weight for a period of 6 weeks.

Collection of Urine sample

At the end of the experimental period the urine sample was collected on ice was free from faecal contamination. Urine sample were centrifuged and aliquots separated. One portion was acidified with concentrated hydrochloric acid and used for the analysis of urea, uric acid and creatinine and the remaining was dialysed at 4°C against distilled water for 3h and later was used for the determination of various enzymes.

Collection of Tissue sample

After the experimental period the animals were sacrificed by cervical dislocation. Blood samples were collected in tubes containing anticoagulant (EDTA) and in tubes without (EDTA). Plasma and serum were separated by centrifugation and was analysed for various parameters. Kidney samples were collected.

Known amount of tissues were homogenized in 0.1M Tris-buffer (pH 7.4) for enzyme assays.

Estimation of Nucleic Acids

Deoxyribonucleic acid (DNA) was estimated, [14] Ribonucleic acid RNA was estimated.[15]

Biochemical Estimation

Estimation of Transaminases and Phosphatases

Aspartate transaminase (AST) and Alanine transaminase (ALT) was estimated[16]. Alkaline phosphatase (ALP) and Acid phosphatase (ACP) were estimated. [17, 18]

Estimation of Marker Enzymes

Estimation of γ-GT.[19] Lactate dehydrogenase (LDH) was estimated.[20] Cathepsin-D activity was estimated.[21] N-acetyl-β-D-glucosaminidase was assayed.[22] B-D-glucuronidase activity was estimated.[23]

RESULTS

Table 1 shows the effect of *T. catappa* L. leaves on body and kidney weight of the control and experimental animals. Body weights were significantly decreased in group II nephrotoxicity induced animals when compared to control group I animals ($p<0.05$), which was significantly increased in drug treated animals group III animals ($p<0.05$) when compared with group I animals. On the other hand, the kidney weights were increased slightly in group II animals ($p<0.05$) when compared to the control animals.

There was a mild decrease in the kidney weight of group III drug treated animals. On the contrary, no statistical differences were observed in kidney weight of group IV drug control animals when compared with group I animals.

Table 1: Effect of *T. catappa* L. leaves on body and kidney weight of control and experimental animals

Particulars	Body weight (gm)	Kidney Weight (gm)
Group I Control	184.01±0.10	0.91±0.88
Group II Pb	144.10±1.13[a]	1.60±1.88[a]
Group III Pb+ *T.catappa*	179.38±0.22[a,b]	1.10±0.97[a,b]
Group IV *T.catappa*	184.18±0.25[b,c]	0.98±0.21[b,c]

Values are expressed as mean + SD for six animals in each, group a - Group I Vs Group II, III and IV,

b - Group II Vs Group III and IV, c - Group III Vs Group IV, The significance at the level of $p<0.05$

The levels of nucleic acids (DNA and RNA) in kidney of control and experimental animals were presented in table 2. In group II animals, the levels of nucleic acids were significantly decreased ($p<0.05$). These were significantly increased in drug treated animals ($p<0.05$). However, no significant changes were observed in group IV drug control animals when compared to the control group I animals.

Table 2: Levels of nucleic acids in kidney of control and experimental animals

Particulars	DNA (gm/g wet tissue)	RNA (gm/g wet tissue)
Group I Control	5.21±0.53	4.40±0.56
Group II Pb	3.13±1.18[a]	2.90±0.51[a]
Group III Pb+ *T.catappa*	4.50±0.34[a,b]	3.99±0.47[a,b]
Group IV *T.catappa*	5.18±0.51[b,c]	4.35±0.21[b,c]

Values are expressed as mean + SD for six animals in each group a - Group I Vs Group II, III and IV,

b - Group II Vs Group III and IV, c - Group III Vs Group IV, The significance at the level of $p<0.05$

Table 3 depicts the activities of transaminases and phosphatases enzymes in the serum of control and experimental animals. The activities of transaminases and phosphatase enzymes such as aspartate transaminase (AST), alanine transaminase (ALT), acid phosphatase (ACP) and alkaline phosphatase (ALP) were significantly increased ($p<0.05$), in lead treated group II animals, when compared with group I animals. On the other hand, drug treated group III animals the activities of these transaminases and phosphatases were decreased when compared with group II animals. No significant changes in these enzymes were observed in Group IV drug control animals when compared with Group I control animals.

Table 3: Activities of *T. catappa* L. leaves on transaminases and phosphatases in serum of control and experimental animals

Particulars	AST (µ moles of pyruvate liberated / mg protein/min)	ALT (µ moles of pyruvate liberated / mg protein/min)	ALP (µ moles of phenol liberated / mg protein/min)	ACP (µ moles of phenol liberated / mg protein/min)
Group I Control	25.10±0.14	20.45±1.21	19.48±1.11	16.33±1.25
Group II Pb	31.17±1.25[a]	27.63±0.16[a]	29.15±1.08[a]	24.71±1.20[a]
Group III Pb+ *T.catappa*	27.12±0.33[a, b]	22.80±1.12[a,b]	21.60±1.18[a ,b]	19.35±1.05[a, b]
Group IV *T.catappa*	25.32±0.34[b, c]	20.71±1.05[b ,c]	19.71±1.00[b, c]	15.99±1.38[b, c]

Values are expressed as mean + SD for six animals in each, group a - Group I Vs Group II, III and IV

b - Group II Vs Group III and IV, c - Group III Vs Group IV, The significance at the level of $p<0.05$

Table 4 shows the activities of transaminases and phosphatases in the kidney of control and experimental rats. The enzymes such as aspartate transaminase (AST), alanine transaminase (ALT), were increased significantly ($p<0.05$) and acid phosphatase (ACP) and alkaline phosphatase (ALP) were significantly decreased ($p<0.05$), in the kidney of group II animals, when compared with group I animals. On the other hand, drug treated group III animals the activities of these enzymes were brought back to near normal when compared with group II animals. No remarkable changes were noticed in Group IV drug control animals when compared with Group I control animals.

Table 4: Activities of *T. catappa* L. leaves on transaminases and phosphatases in kidney of control and experimental animals

Particulars	AST (µ moles of pyruvate liberated / mg protein/min)	ALT (µ moles of pyruvate liberated / mg protein/min)	ALP (µ moles of phenol liberated / mg protein/min)	ACP (µ moles of phenol liberated / mg protein/min)
Group I Control	14.08±0.11	10.44±0.91	2.44±1.01	7.80±0.22
Group II Pb	18.09±0.50[a]	13.33±1.11[a]	4.15±0.18[a]	9.66±0.60[a]
Group III Pb+ *T.catappa*	16.77±1.43[a, b]	11.10±1.02[a,b]	3.56±0.21[a, b]	8.30±1.15[a, b]
Group IV *T.catappa*	13.99±1.12[b, c]	10.22±0.05[b, c]	2.35±0.10[b, c]	7.66±0.26[b, c]

Values are expressed as mean + SD for six animals in each group a - Group I Vs Group II, III and IV

b - Group II Vs Group III and IV, c - Group III Vs Group IV, The significance at the level of $p<0.05$

Table 5 represents the levels of lysosomal marker enzymes such as γ-GT, β-D- glusosaminidase, N-acetyl-β-D-glusosaminidase, Cathepsin-D and LDH in the urine of control and experimental animals. Group II animals showed a significant increase in the levels of these brush border marker enzymes in the urine may be due to renal cellular damage in kidney on lead induction ($p<0.05$). The levels of these lysosomal marker enzymes were declined significantly in aqueous extract of *Terminalia catappa* L leaves treated group III animals when compared with group II lead intoxicated animals ($p<0.05$). No remarkable changes were observed in group IV drug control animals when compared with control animals.

Table 5: Levels of marker enzymes in urine of control and experimental animals

Particulars	γ-GT (IU/L)	β-D- glusosaminidase (µ moles of p-nitrophenol liberated / mg protein/min)	N-acetyl-β-D-glusosaminidase (NAG) (µ moles of phenol liberated / mg protein/min)	Cathepsin-D (nmoles tyrosine formed/hr/mg protein)	LDH (µ moles of pyruvate liberated / mg protein/min)
Group I Control	2.99±0.11	0.55±0.08	0.60±1.02	0.05±0.04	0.25±1.22
Group II Pb	4.87±1.12[a]	0.89±0.12[a]	1.65±0.04[a]	0.84±1.20[a]	1.45±0.36[a]
Group III Pb+ *T.catappa*	3.44±1.65[a, b]	0.64±1.09[a, b]	0.71±1.23[a, b]	0.55±0.05[a, b]	0.69±1.43[a, b]
Group IV *T.catappa*	2.87±1.22[b, c]	0.54±0.54[b, c]	0.59±0.06[b, c]	0.04±1.44[b, c]	0.21±0.56[b, c]

Values are expressed as mean + SD for six animals in each group

a - Group I Vs Group II, III and IV, b - Group II Vs Group III and IV, c - Group III Vs Group IV

The significance at the level of $p<0.05$

Table 6 illustrates the levels of marker enzymes in the kidney of control and experimental animals. The activities of marker enzymes such as γ-GT, β-D- glusosaminidase, N-acetyl-β-D-glusosaminidase, Cathepsin-D, and LDH were decreased significantly in group II lead injected animals when compared with group I control animals ($p<0.05$). The levels of these marker enzymes were increased significantly in aqueous extract of *T.catappa* L leaves treated group III animals when compared with group II animals ($p<0.05$). No noticeable changes were observed in group IV animals when compared with group I control animals.

Table 6: Levels of marker enzymes in kidney of control and experimental animals

Parameters	γ-GT (IU/L)	β-D- glusosaminidase (µ moles of p-nitrophenol liberated / mg protein/min)	N-acetyl-β-D-glusosaminidase (NAG) (µ moles of phenol liberated / mg protein/min)	Cathepsin-D (nmoles tyrosine formed /hr/mg protein)	LDH (µ moles of pyruvate liberated / mg protein/min)
Group I Control	1.70±1.01	1.54±0.44	1.84±0.18	3.15±0.08	1.41±1.03
Group II pb	0.78±0.11[a]	0.99±0.02[a]	1.09±1.40[a]	1.99±1.20[a]	0.66±1.16[a]
Group III pb+ *T.catappa*	1.24±0.76[a, b]	1.29±0.08[a, b]	1.40±0.06[a, b]	2.55±0.09[a, b]	1.13±0.15[a, b]
Group IV *T.catappa*	1.69±0.06[b, c]	1.50±0.15[b, c]	1.81±1.17[b, c]	3.08±0.18[b, c]	1.40±0.61[b, c]

Values are expressed as mean + SD for six animals in each group

a - Group I Vs Group II, III and IV, b - Group II Vs Group III and IV, c - Group III Vs Group IV

The significance at the level of $p<0.05$

DISCUSSION

The present investigation attempts to evaluate protective efficacy of *Terminalia catappa* leaves against lead induced nephrotoxicity in *in vivo* model. The increase in kidney weight may be due to renal tissue fluid retention and a significant decrease in body weight in lead intoxicated animals due to oxidative stress caused by lead.[24] Sanchez-Fructuoso *et al.,* [25] suggests that it is noticeable that exposure of about 0.5% lead acetate for a period of 90 days resulted in increased levels of lead in urine and tissue levels in experimental rats. *Terminalia catappa* decrease the kidney weight and increase the body weight by reducing the oxidative stress in blood and renal tissues formed due to lead induction. Previous studies showed that the water extract of *T. catappa* L. leaves exert antioxidative activities.[26] It is well known fact that proteins, lipids and DNA are the major targets of oxidative injury.[27] DNA and RNA damage may be associated with the production of free radicals caused by lead.[28] In the present investigation decreased levels of nucleic acids were observed in kidney of the lead treated animals. Hence nucleic acids are sensitive to free radicals induced by lead. The observed decreased levels of DNA and RNA in lead induced animals may be due to its inhibitory effects on the DNA synthesis and RNA transcription in tissues. Treatments with T*erminalia catappa* leaves extract probably may prevent lead induced UTP depletion and a subsequent suppression of nucleic acids by restricting the formation of toxic metabolites formed by lead.

Aminotransferases like ALT and AST being an important class of enzymes regarded as markers of liver injury and linking carbohydrate and amino acid metabolism.[29] Sivalokanathan *et al.,* [30] have reported that increased levels of AST and ALT were indicators of cellular damage and loss of functional integrity of cell membrane and thus leading to release of enzymes into blood circulation. High levels of transaminases released into blood may be an indicator of liver, kidney and other tissue damage.[31] In the present investigation the activities of alanine and aspartate amino transaminases were found to be significantly increased in lead intoxicated animals. Thus it was suggested that the alteration in these enzyme levels in serum might be due to the biotransformatic products of lead could have affected the serum, liver and kidney. Free radical scavenging activity of flavonoids present in medicinal plants had prevented the cellular damaged.[32] Therefore in this context, the flavonoids present in the T*erminalia catappa* leaves extract might have ameliorated the transaminases and thereby prevented the cellular damaged caused by lead.

Phosphatases are a group of non-specific phosphomonoseterases that involved in the hydrolyase asters of orthophosphates in alkaline (ALP) or acidic condition (ACP). They are also implicated in transport of metabolites across the cell membranes, protein synthesis, secretary activities, synthesis of certain enzymes and glycogen metabolism.[33] ALP, a membrane bound enzyme, has its high activity in the brush-border membrane and its alterations is likely to affect the membrane permeability and produce derangements in the transport of metabolites.[34] Reduced ALP activity in renal tissue may be due to altered synthesis of secretary enzymes.[35] ACP is a lysosomal enzymes present in body fluids and tissues, which is a responsive serum and tissue marker in detecting of various disorders.[36] The elevated levels of phosphatases may be due destruction of cell membrane further leads to liberation of degradative enzyme. The newly synthesized enzyme more easily cross the damaged membrane and appear in serum.[37] Gill *et al.,* [38] reported that serum and liver ACP increased in lead treated rats, and in renal tissue it was decreased marginally *in vivo* which clearly indicates that lead acetate can damage kidney both structurally and functionally. The phosphatases were reverted to normal level by treatment with aqueous extract of T*erminalia catappa* leaves which indicates the rationale for the use of T*erminalia catappa* as a suitable herbal treatment for hepatic and renal disorders.

Marker enzymes sensitively reflect the significance of the liver damage. Due to the alternations in the permeability of the plasma membrane, the enzymes are leaked and it results in an increase in their serum level. [39] N-acetyl-β-D-glusosaminidase (NAG) a sensitive indicator of tubular damage lysosomal enzymes located in both proximal and distal tubules which are released after renal tubular damage.[40] This may be due to membrane damage of lysosomes accumulating myeloid body formation and subsequent release of hydrolytic lysosomal enzymes such as NAG, β-D- glusosaminidase and cathepsin-D into cytosol which may lead to renal tubular cell necrosis.[41] Cathepsin-D is a ubiquitous partly endoprotease which is involved in normal protein degradation with lysosomes.[42] This protease appears to be a useful marker in renal damage. γ-GT is only one of a number of renal enzymes that have been systematically studied in urine sample as a potential marker of nephrotoxic and ischemic renal injury in experimental animals and in humans.[43] The investigation of LDH, a regulator of many biochemical functions in the body and fluids is distributed over most parts of the nephron and has proved to be most sensitive in a variety of experimental nephropathies. Increased excretion of LDH in urine occurs after exposure to lead acetate along with a profound increase in the kidney weights.[44] Urinary enzymes levels have been shown to elevated in a wide spectrum of renal diseases.[45] Changes in urinary and renal tissue enzymes may be due to one or a combination of factors including increased plasma levels or decreased reabsorption of the compounds as well as structural lesions in the nephron.[46] In the present investigation there was a significant decrease in lysosomal marker enzymes in kidney and there was an increase in urine samples which clearly indicating the nephrotoxicity induced by lead. Treatment of T*erminalia catappa* maintains nephrotoxicity suggesting, an increase in the stability of lysosomal and brush border membrane of proximal tubular cells, the important sites for capacity of lysosomal to release hydrolytic enzymes.

CONCLUSION

From the results of our present investigation, it was concluded that aqueous extract of T*erminalia catappa* L leaves can be more effective and have a protective role against lead induced nephrotoxicity.

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