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Effect of *Chromolaena odorata* and *Boerhavia erecta* on lipid peroxidation and antioxidants of Lead-acetate induced Nephrotoxicity in rats

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ABSTRACT

The aim of the present study was to evaluate the effect of *Chromolaena odorata (C.odorata)* and *Boerhavia erecta (B.erecta)* on lipid peroxidation and antioxidants in Lead acetate-induced Nephrotoxicity in male Wistar rats. The rats of the nephrotoxicity control group 2 were administered lead-acetate 10mg/kg body weight, intraperitoneally in 3 days once for 15 days. In nephrotoxicity rats, Oral treatment with *C.odorata, B.erecta* at a daily dose of (500 mg/kg b.wt) and silymarin (25 mg/kg b.wt) groups (3, 4 and 5) for last 15 days. The Lead-acetate-induced rats showed increase in the level of lipid peroxidation and decreased levels of superoxide dismutase, catalase, glutathione peroxidase and reduced glutathione in serum and kidney. Oral treatment with *C.odorata, B.erecta* and silymarin in nephrotoxicity induced rats showed significantly decrease in the levels of kidney and serum lipid peroxidation and significant ($p<0.05$) increase in the levels of superoxide dismutase, catalase, glutathione peroxidise and reduced glutathione in kidney and serum. The study results revealed that *C.odorata, B.erecta* ameliorates kidney damage in lead-acetate induced nephrotoxicity, by maintaining lipid peroxidation and antioxidants levels by its free radical scavenging effects on lead-acetate induced nephrotoxicity rats.

Keywords: Lipid peroxidation, antioxidants, *Chromolaena odorata* , *Boerhavia erecta*.

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INTRODUCTION

Nephrotoxicity is one of the most common kidney problems and occurs when the body is exposed to a drug or toxic metal that causes damage to the kidneys. Environmental pollution is the presence of a pollutant in environment such as air, water, soil and consequently in food which may be poisonous or toxic and will cause harm to living things in the polluted environment [1]. The excessive amount of pollutants such as heavy metals in animal feed and feed stuffs are often due to human actions and they result from either agricultural or industrial production or through accidental or deliberate misuse [2, 3, 4, 5].

Lead is known to induce a broad range of physiological, biochemical and behavioral dysfunction in laboratory animals and humans [6]. 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.

Silymarin is a flavonoid extracted from silybum marianum, that has already successfully been applied as a protective agent in various clinical and both *in vivo* and *in vitro* experimental models of hepatotoxicity [7, 8] and nephrotoxicity to a certain extent [9.]

Medicinal plants have been used by all civilization as a source of medicines since ancient times. In recent times, there has been growing interest in exploiting the biological activities of different ayurvedic medicinal herbs, due to their natural origin, cost effectiveness and lesser side effects [10]. Interest in medicinal plants as a re-emerging health aid in the maintenance of personal health and well-being has been fuelled by rising costs of prescription drugs, and the bioprospecting of new plant-derived drugs [11].

Chromolaena odorata (L.) King and Robinson (Asteraceae, Eupatorieae), known as siam weed. It is used as remedy for variety of ailments, including malaria, fever, infections and as haemostatic agent. It also possesses anti-fungal and anti-bacterial properties [12].

Boerhavia erecta is a weedy herb of the family Nyctaginaceae and is commonly available in almost all places. It is used as a traditional medicinal plant in Africa [13]. It has been found to possess diuretic action, anti-inflammatory, antifibrinolytic, anticonvulsant and hepatoprotective activities [12].

Therefore the present study was to evaluate the nephroprotective role of *C.odorata* and *B.erecta* against lead acetate-induced nephrotoxicity in rats.

MATERIALS AND METHOD

Chemicals

Lead-acetate was purchased from the Sigma Chemical Co. (St. Louis, MO, USA). All the other chemicals and reagents used were of analytical grade.

Plant Materials

The leaves of *C.odorata* and *B.erecta* were collected from Kolli Hills in Namakkal District, Tamilnadu, India. The plants were identified at the plant anatomy research centre. A voucher specimen of the plants has been deposited (Accession No: PARC/2012/1389 and PARC/2012/1388). The samples were shade dried at room temperature and then ground to a fine powder in a mechanic grinder. The powdered materials were then extracted by the solvent ethanol in the ratio 1:10 using soxhlet apparatus. After extracting all colouring materials the solvent was removed by evaporating on water bath which give rise to a solid mass of the extract. These extracts dissolved in water before oral administration to the experimental animals.

Induction of Nephrotoxicity

The nephrotoxicity was induced by intraperitoneal (i.p.) injection of lead-acetate (10 mg/kg body weight) dissolved in double distilled water, 3 days once for 15 days.

Formulation of silymarin

The silymarin (25 mg/kg body weight) was suspended in distilled water and administrated by intragastric intubation.

Animal Housing and Diets

Male Wistar albino rats weighing about 150g were obtained from Sri Venkateshwara enterprises Bangalore, India. After one week of acclimatization all animals were housed six per polypropylene plastic cage covered with metal grids and a hygienic bed of husk in a specific-pathogen free animal room under controlled conditions of a 12h light/12 hour dark cycle, and provided with standard food pellets (diet composition, wheat broken-moisture 9.0%, crude protein, 11.5% crude fat, 1.9% crude fibre 4% ash 0.2%, nitrogen-free extract 73.4%) supplied by Hindustan Lever Ltd, Mumbai, India) and tap water *ad libitum.* Animals were maintained as per the principles and guidelines of the Ethics Committee of Animal Care of Periyar University in accordance with the Indian National Law on animal care and use (Reg.No. PU – IAEC(1085/ac/07).

Study Design and Treatment schedule

The rats were randomly assigned into five groups of 6 animals each. Total duration of the experiment was 30 days.

Group I : Control

Group II : Lead-acetate (nephrotoxicity) induced rats (10 mg /kg/b.wt.) 3 days once for first 15 days.

Group III : Lead- acetate (nephrotoxicity) induced rats (10 mg /kg/b.wt.) 3 days once for first 15 days. + *C.odorata* (500 mg/kg/b.wt) for last 15 days.

Group IV : Lead- acetate (nephrotoxicity) induced rats (10 mg /kg/b.wt.) 3 days once for first 15 days. + *B.erecta* (500 mg/kg/b.wt) for last 15 days.

Group V : Lead- acetate (nephrotoxicity) induced rats (10 mg /kg/b.wt.) 3 days once for first 15 days. + Silymarin (25 mg/kg b.wt.) for last 15 days.

At the end of the experimental period, rats were sacrificed by cervical decapitation. The blood was collected and serum obtained after centrifugation were used for various biochemical estimations. Kidneys were removed, cleared of blood and immediately transferred to ice cold containers containing 0.9% sodium chloride. Samples of tissues were homogenized in appropriate buffer and used for the determination of the following parameters.

Preparation of tissue homogenate

Kidney tissue were removed immediately and washed with ice-cold saline and homogenized in the appropriate buffer in a tissue homogenizer.

Biochemical estimations

Lipid peroxidation was estimated by measuring the level of thiobarbituric acid reactive substances (TBARS) in tissues via the method Niehaus and Samuelson [14]. Reduced glutathione (GSH) content was determined via the method Moron et al. [15]. Glutathione peroxidase (GPx EC.1.11.1.9) activity was assayed via the method Rotruck, [16]. Superoxide dismutase (SOD EC.1.15.1.1) was assayed using the method Marklund and Marklund [17].The activity of catalase (CAT EC. 1.11.16) was determined via the method Sinha [18].

Histopathological examination

After the experimental period, animals were decapitated, and their kidneys were removed immediately, then sliced, and washed in saline. For histopathological analysis, kidney specimens fixed in 10% formalin were embedded in paraffin, sliced at 5-mm thickness, and stained with hematoxylin and eosin for detection of kidney damage. The pathological changes were assessed and photographed.

Statistical analysis

The results presented here are the means ± SD of 6 rats in each group. The results were analyzed using one-way analysis of variance [ANOVA and the group means were compared using Duncan's multiple range test [DMRT] using SPSS version 16 for Windows. The findings were considered as statistically significant if $P<0.05$ [19].

RESULTS AND DISCUSSION

Histopathological changes in the control and experimental rats

Figure 1 shows the histopathological changes of control and experimental rats. A) Histopathologic sections from control group glomeruli show normal cellularility. The basement membrane is normal. The tubules are lined by normal cuboidal cells. The stroma shows no inflammation. B) Lead acetate group showed extensive renal tubular injury such as tubular cell necrosis, cast formation, loss of brush border, dilatation of tubules and inflammatory cell infiltration. (C, D, E) Lead acetate treated with *C.odorata, B.erecta* and silymarin showed near normal glomeruli and tubules.

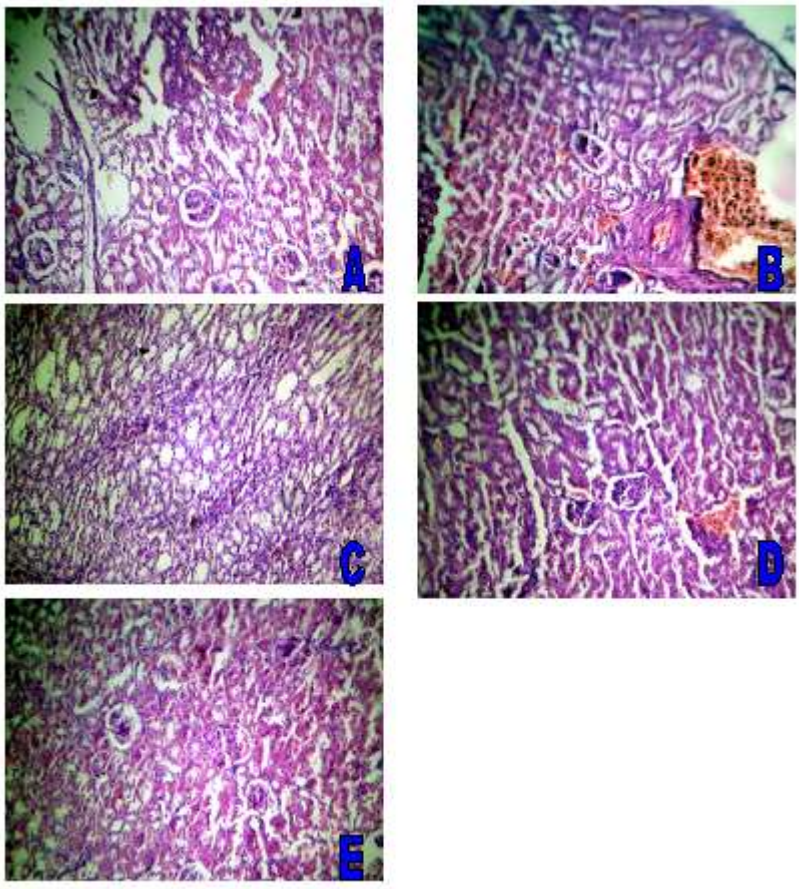

Figure 1: Histopathological changes in the control and experimental rats

. *Effect of C. odorata and B. erecta on lipid peroxidation in control and experimental rats*

Table 1 depicts the effect of *C.odorata, B.erecta* on serum and tissue levels of TBARS in the control and experimental rats. The levels of serum and tissue TBARS was significantly increased in the nephrotoxicity of Lead acetate induced rats (group 2) as compared to the control rats (group 1). However, treatment with *C.odorata* and *B.erecta* reduced TBARS levels to normal levels. *C.odorata, B.erecta* and silymarin administration to lead acetate treated rats (groups 3, 4 and 5)

significantly decreased the levels of TBARS in tissue and serum as compared with nephrotoxicity of Lead acetate-induced rats (group 2).

Effect of C. odorata and B. erecta on serum antioxidants in control and experimental rats

Table 2 illustrates the effect of *C.odorata* and *B.erecta* on serum antioxidants (SOD, CAT, GSH and GPx) in the control and experimental rats. The circulating levels of (SOD, CAT, GSH and GPx) were significantly decreased in the Lead acetate-induced rats (group 2) as compared to the control rats (group 1). *C.odorata*, *B.erecta* and silymarin administration to Lead acetate-induced rats (groups 3, 4 and 5) was significantly increased the levels of antioxidants (SOD, CAT, GSH and GPx) as compared to the Lead acetate-induced rats (group 2).

Table 1: **Effect of *C.orata* and *B.erecta* on serum and tissue lipid peroxidation (TBARS) of control and experimental rats**

Groups	**Control**	**Lead acetate (10 mg/kg b.wt)**	**Lead acetate + *C. odorata* (500 mg/ kg b.wt)**	**Lead acetate + *B. erecta* (500 mg/ kg b.wt)**	**Lead acetate +Silymarin (25 mg/kg b.wt)**
serum thiobarbituric acid reactive substances (nmol/ml)	1.8± 0.34[b]	5.8±0.29[a]	4.11±0.42[b]	4.2±0.39[a]	3.9±0.39[e]
Kidney tissue thiobarbituric acid reactive substances (nmol/mg protein)	4.6±1.80[a]	10.1±0.54[d]	7.88±0.56[c]	8.19±0.29[b]	7.1±1.02[e]

The values are expressed as mean ±SD of six rats in each groups. Values marked with different letter (a-e) differ significantly at ($p<0.05$)

Table 2: Effect of *C. odorata* and *B.erecta* on serum antioxidants of control and experimental rats.

Groups	**Control**	**Lead acetate (10 mg/kg b.wt)**	**Lead acetate + *C. odorata* (500 mg/ kg b.wt)**	**Lead acetate + *B. erecta* (500 mg/ kg b.wt)**	**Lead acetate + Silymarin (25 mg/kg b.wt)**
Superoxide dismutase (SOD) (nmoles of H_2O_2 utilized/min/mg protein)	5.2± 2.34[a]	2.9±0.29[a,c]	3.85±0.42[b]	3.72±0.39[d]	4.24±1.39[c]
Catalase (CAT) (enzyme required for 50% inhibition of NBT reduction)	68±1.80[a]	45.5±2.54[a]	57.1±1.56[b,c]	59±2.29[b]	61.1±3.80[d]
Glutathione peroxidase (GPx) (nmoles g-1 wet tissue)	8.33±1.03[a]	2.76±0.02[c]	6.28±0.026[c]	6.19±0.027[b]	6.33±1.03[a,d]
Glutathione reductase (nmoles GSH oxidized min-1 mg-1 protein)	4.8±1.58[b]	2.11±0.78[d]	3.62±0.91[b]	3.55±0.22[b,c]	3.81±1.17[e]

The values are expressed as mean ±SD of six rats in each groups. Values marked with different letter (a-e) differ significantly at ($p<0.05$)

Table 3: Effect of *C. odorata* and *B. erecta* on tissue antioxidants of control and experimental rats.

Groups	**Control**	**Lead acetate (10 mg/kg b.wt)**	**Lead acetate + *C. odorata* (500 mg/ kg b.wt)**	**Lead acetate + *B. erecta* (500 mg/ kg b.wt)**	**Lead acetate + Silymarin (25 mg/kg b.wt)**
Superoxide dismutase (SOD) (U/mg protein)	8.41± 0.45[c]	6.73±0.35[a]	7.71±0.55[b]	7.80±0.60[b]	7.66±0.43[b]
Catalase (CAT) (U/mg protein)	20±1.58[c]	15.51±0. .76[a]	17.91±0. 38[b]	18.31±0. 54[b]	18.63±0.69[b]
Glutathione peroxidase (GPx) (U/mg protein)	133±3.03[c]	76±4.02[a]	108±3.06[b]	113±4.27[b]	119±3.12[a,c]
Reduced glutathione (n moles/ mg protein)	18.93±0.43[d]	9.58±0.64[a]	14.31±0.67[b]	14.03±0.89[b]	15.15±0.81[c]

The values are expressed as mean ±SD of six rats in each groups. Values marked with different letter (a-d) differ significantly at ($p<0.05$)

Effect of C. odorata and B. erecta on tissue antioxidants in control and experimental rats

Table 3 depicts the effect of *C.odorata* and *B.erecta* on kidney tissue antioxidants (SOD, CAT, GSH and GPx) of control and experimental rats. The tissue antioxidant levels (SOD, CAT, GSH and GPx) were significantly decreased in nephrotoxicity of Lead acetate-induced rats (group 2) as compared to the control rats (group 1). *C.odorata, B.erecta* and silymarin administration to nephrotoxicity of Lead acetate-induced rats (groups 3, 4 and 5) were significantly increased the levels of tissue antioxidants (SOD, CAT, GSH and GPx) as compared to the Lead acetate-induced rats (group 2).

DISCUSSION

Nowadays, lead (Pb) is an ubiquitous environmental contaminant found in air, canned food, drinking water, and paints [20] due to its significant role in modern industry [21]. Lead toxicity is closely related to its accumulation in certain tissues, and its interference with the bioelements whose role is critical for several physiological processes. Pb has been found to produce wide range of biochemical and physiological dysfunctions in humans and laboratory animals [22].

Free radicals and reactive oxygen species mediate the propagation of peroxidation of polyunsaturated fatty acids; this cascade can be prevented through enzymatic and nonenzymatic antioxidants. Lipid peroxidation is regarded as one of the basic mechanisms of tissue damage caused by free radicals [23].

Lead is known produces oxidative damage in liver and kidney tissue by enhancing peroxidation of membrane lipids, a deleterious process solely carried out by free radicals [24]. In the present study, the increased in the lipid peroxidation was assessed by the elevated levels of TBARS following lead acetate administration. Lipid peroxidation will inactivate cell constituents by oxidation and ultimately lead to loss of membrane integrity [25, 26] and it may also due to the formation of free radicals or through exhaustion of antioxidants, leading to oxidative stress. Decreased levels of TBARS with *C.odorata* and *B.erecta* in lead acetate treated animals obtained in this study indicates the ameliorating effects of *C.odorata* and *B.erecta* against the oxidative stress induced with lead acetate in kidneys.

Antioxidants are widely used to prevent oxidative stress in the cells and most of them are metabolized in the liver and the kidney. To prevent oxidative damage in the cells, a variety of antioxidants are used as scavenger of free radicals [27]. Antioxidant enzymes, such as including catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) are an important

antioxidant defense in cells exposed to oxygen. They can manage with ROS generated and could be vital in protecting the cell against free radical damage.

In the present study administration of lead acetate shows decreased levels of SOD, GPx and CAT. These changes could lead to the generation of reactive oxygen species (ROS) or by reducing the antioxidant cell defense system by depleting glutathione or by inhibiting sulfhydryl dependent enzymes or by interfering with some essential metals (copper) needed for antioxidant enzyme activities and by increasing cell susceptibility to oxidative attack by altering the membrane integrity and faulty acid composition [28]. In the present study we observed, decreased in the level of SOD, CAT, GPx and GSH in lead acetate induced rats (Group 2) as compared to control rats. It may be due to oxidative stress. Treatment with *C.odorata* and *B.erecta* increased the activity of SOD, CAT, GPx and GSH, it may be due to higher production of H_2O_2.

GSH, a non-enzymatic antioxidant, plays pivotal role in detoxification processes including conjugation of reactive intermediates and maintenance of GSH redox cycle as antioxidant armory, thus protecting oxidative stress [29]. It reduces hydrogen peroxides and hydroperoxides by its redox and detoxification reactions, and protects protein thiol groups from oxidation. The administration of *C.odorata* and *B.erecta* ethanolic extracts at 500 mg/kg of body weight preserved GSH levels, it may be due to indirectly induces the biosynthesis of GSH and provides a protective intracellular mechanism, presumably as a free radical scavenger for toxic agents. Hence, we can speculate that the *C.odorata and B.erecta* extracts in our study may induce the synthesis of endogenous GSH, which protects against Lead acetate-induced oxidative damage.

Histopathological investigations revealed that lead acetate exposure caused progressive glomerular and tubular alterations. These findings are agreement with results of previous investigations by Ahemd et al., [30]. Who recorded alterations in renal histopathology after environmental exposure to lead acetate. Tubular vacuolization, necrosis and dilation found in the present studies due to lead intoxication were reported previously by Karmakar et al., [31]. These tubular alterations caused by lead acetate toxicity might be a result of a hydrolic changes in the renal tissue and suggest that lead intoxication yields to a partial failure in the ions pump transport tubules cells which in turn produces tubular swelling and causes necrosis and vacuolization of the tubules. *C.odorata and B.eracta* could improve to some extent the altered kidney histopathology.

CONCLUSION

This study demonstrates that *C.odorata, B.erecta* has protective effects against nephrotoxicity induced by lead acetate in rats. The mechanisms which contribute to its effectiveness involve the

quenching of free radicals, increasing antioxidant status. According to our biochemical findings, which were supported by histopathological evidence, administration of *C.odorata, B.erecta* abolished nephrotoxic effects of Lead acetate. This study substantiated the scientific evidence in favors of its pharmacological use in renal injuries in folk medicine.

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