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Research Article

PROTECTIVE EFFECT OF *ORTHOSIPHON STAMINEUS* LEAVES AGAINST LEAD ACETATE AND CADMIUM CHLORIDE INDUCED RENAL DYSFUNCTION IN RATS

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ABSTRACT

Though our previous study has showed that pretreatment with *Orthosiphon Stamineus* methanol extract (OSM) prevents gentamycin induced nephrotoxicity. No report has been documented on the effect of Pretreatment of OSM with Cadmium and Lead. Therefore this study aims to investigating the effect of pretreatment with OSM on Cadmium and Lead induced Nephrotoxicity. Nephrotoxicity was induced in Wistar rats by intraperitoneal administration of Lead Acetate 8mg/kg/day for 21 days and oral administration of Cadmium Chloride 50mg/kg for a single day. Effect of concurrent administration of *Orthosiphon Stamineus* leaf extract at a dose of 100mg/kg and 200 mg/kg/day given by oral route was determined using serum and urinary creatinine and blood urea and Uric acid as indicators of kidney damage. The present work demonstrates that Rat chronically intoxicated with two heavy metals display a pronounced impairment in kidney function which is confirmed by the enhancement of plasma creatinine, urea and uric acid levels, and histopathological alterations. The results of the present work showed that the cortex is more affected than the medulla due to long-term treatment with heavy metals. This could be partly due to uneven distribution of heavy metals in the tissue of the kidney where about 90% of the total renal blood flow enters the cortex via the bloodstream. *Orthosiphon Stamineus* Leaf extract 200mg/kg normalized the Cadmium Chloride and Lead Acetate induced increases in urine and plasma creatinine, and blood urea levels. It was observed that the Methanol extract of *Orthosiphon Stamineus* leaves 200mg/kg significantly protects rat kidneys from Cadmium Chloride and Lead Acetate –induced Nephrotoxicity. *Orthosiphon* Leaves has protective effect on lead and cadmium induced renal toxicity.

Keywords: Lead acetate; Cadmium chloride; *Orthosiphon stamineus*; Renal protection; Leaves

INTRODUCTION

Cadmium is a common nephrotoxic agent in food and tobacco [2,9]. Other major sources of cadmium are cereals, vegetables and shell fish. Cadmium mainly accumulates in the kidneys and liver. Cadmium accumulates in the renal cortex and induces tubular toxicity [3]. Cadmium obtained as a by-product of zinc refining, is used industrially in plating of steel, pigments, plastics, alloys, and nickel-cadmium batteries, and in nuclear and electronic engineering [1,8]. Because the biologic half-life of cadmium is long (more than 30 yr), prolonged low level exposure leads to excessive accumulation in certain tissues, especially the kidney. Studies of cadmium toxicity have been performed in the accumulator battery industry, the copper-cadmium alloy industry, and cadmium pigment factories. Chronic poisoning typically was found to have occurred after several years of exposure and was characterized clinically by variable features of naso respiratory involvement, such as emphysema, rhinitis, alteration of nasal mucosa. Yellow tooth discolouration, mild anaemia, disturbances in calcium metabolism, osteomalacia, and renal toxicity also were observed occasionally. Environmental cadmium exposure occurs in residents living in proximity to industrial pollution and also in heavy smokers, as tobacco smoke yields high cadmium concentrations. Hence the present study was undertaken to investigate the nephrotoxic effects of cadmium chloride and lead acetate on kidneys of Male wistar Rats and also to monitor the protective effect of *Orthosiphon stamineus* on cadmium chloride and lead acetate induced nephrotoxicity on the basis of histopathological observations.

Lead (Pb) is a poisonous metal, which is ubiquitous in both organic (Tetraethyl lead) and inorganic (lead acetate, lead chloride) forms in environment[15] and is emitted from automobile fuels, industrial discharge and paints [11] Workers employed in these industries are more exposed to lead than general public[10] Lead has been known as an important environmental toxicant for many years. Lead poisoning is involved in the structural and functional abnormalities of multiple organ systems. Lead is a non-threshold multi-targeted toxicant that causes alterations in different organs of the body, including the kidney. The absorbed lead is conjugated in the liver and passed to the kidney, where a small quantity is excreted in urine and the rest accumulates in various body organs and interferes with their functions, specially the kidney as a target site for lead toxicity. Lead many undesired effects, including neurological, Behavioral ,renal , hepatic and reproductive dysfunctions [16]

Orthosiphon Stamineus commonly known as Misai kucing and widely grown in Southeast Asia . Leaves of this plant are used commonly in Southeast Asia for herbal tea, well known as “Java tea”. Leaves of this plant have been used to treat rheumatism, abdominal pain, kidney and bladder inflammation, edema, gout and hypertension. Scientific studies have found that the leaves exhibit dynamic pharmacological properties such as, antioxidant, antibacterial, heptoprotective, anti-inflammatory, cytotoxic, diuretic and antihypertensive properties [5,6,16]

Though our previous study has showed that pretreatment with *Orthosiphon Stamineus* prevents Cisplatin induced nephrotoxicity .No report has been documented on the effect of Pretreatment of OSM with Cadmium and Lead. Therefore this study aims to investigating the effect of pretreatment with OSM on Cadmium and Lead induced nephrotoxicity.

In the present experiment the animals were divided in to nine groups, each group comprised of 6 Rats were maintained in plastic cages. The first group which served as control group was administered only vehicle (water). The second and third experimental group were administered only OSM extract (100 and 200 mg/day/animal) for 16 days. The Fourth experimental group was administered only single oral dose of

cadmium chloride (50mg/kg/animal/day). The fifth and sixth experimental groups were pretreated with OSM extract for 15 days with a dose of 100 and 200 mg/day/animal and on 16th day was administered cadmium chloride (50mg/kg/animal/day). Histopathological observations of kidney in experimental Rats, administered single dose of CdCl2 distinctly revealed renal damage which was evident from significantly increase in number of degenerated and desquamation of cells in renal tubules, induction of sclerotic glomeruli. The histoarchitecture of glomeruli and renal tubules in animals co administered OSM extract and cadmium chloride showed significant decrease in number of degenerated renal tubules, desquamation of cells was less than only cadmium treated group and occurrence of normal glomeruli were seen. Hence it can be propounded that *Orthosiphon Stamineus* leaves can serve to check nephrotoxic effects of cadmium chloride.

MATERIALS AND METHODS

The study was conducted after taking prior approval by Institutional Animal Ethical Committee (1012 /c/06/CPCSEA), on twenty four adult male Swiss albino mice 32-50 days old and weighing around to 30-40g.These were maintained in plastic cages under controlled lighting conditions (12:12 light: dark cycle) relative humidity (50 ± 5%) and temperature (37 ± 2°C), fed with mice feed and were given ad libitum access to water.

Experimental Design

Nine groups of 6 Rats per experiment were taken and treated with CdCl2,

Lead acetate and *Orthosiphon Stamineus*.. The dose protocol was as follows-

Group-I

A Control group, treated with only vehicle (water) for a day.

Group-II

A Control group treated with only OSM Extract 100mg/Kg/day orally for 16days.

Group-III

A Control group treated with only OSM Extract 200mg/Kg/day orally for 16 days.

Group-IV

Experimental batch, administered CdCl2 50mg/kg/animal orally for a single day.

Group-V

Experimental batch, pre-treated with OSM extract 100mg/animal/day orally for 15 days and on the16th day a single oral dose of CdCl2 50mg/kg/day/ animal was administered.

Group-VI

Experimental batch, pre-treated with OSM extract 200mg/animal/day orally for 15 days and on the16th day a single oral dose of CdCl2 50mg/kg/day/ animal was administered

Group VII

Control group treated with intra peritoneal (i.p.) injection of lead acetate (8 mg/kg) for 21 days.

Group VIII

Experimental batch, pre-treated with OSM extract 100mg/animal/day for 21 days

Group IX

Experimental batch, pre-treated with OSM extract 200mg/animal/day for 21 days.

An hour after the treatment with *Orthosiphon Stamineus*, VIII and IX groups were intra peritoneally injected with lead acetate (8 mg/kg) for 21 days

Twenty-four hours after administration of last dose, the control and the experimental animals were sacrificed. Kidneys were excised and subsequently fixed in Bovins solutions. After fixation kidneys were processed, wax block and slides were prepared then stained in haematoxylin and eosin for histopathological studies.

Bio- Chemical study

Animals were sacrificed by cervical dislocation. The blood samples were collected via retro orbital puncture and the serum was used for the assay of marker enzymes viz: blood urea, serum creatinine were estimated by the method of Brod and Sirota et al [4] and Marshell et al [12] respectively. Levels of reduced glutathione by the method of Moron et al [13] and Superoxide dismutase by Kakkar et al [7] and both kidneys were isolated from each rat. The kidneys were processed for histopathological examination.

Histopathology

The kidneys were sectioned longitudinally in two halves and were kept in 10% neutral formalin solution. Both kidneys were processed and embedded in paraffin wax and sections were taken using a microtome. The sections were stained with hematoxylin and eosin and were observed under a computerized light microsope.

Statistical analysis

The results were expressed as mean ± sem of six animals from each group. The statistical analysis was carried out by one way analysis of variance (ANOVA) P value < 0.05 were considered significant.

TABLE 1: EFFECT OF OSM EXTRACT ON BIOCHEMICAL PARAMETERS IN RATS SUBJECTED TO CADMIUM CHLORIDE INDUCED NEPHROTOXICITY

S.NO	GROUPS	TREATMENT AND DOSE	Blood Uric acid (mg/dl)	Serum Creatinine (mg/dl)	Serum Urea (mg/dl)	Urine creatinine
1	Group I	Control	2.42±0.11	0.37 ± 0.03	30±2.51	96.71±1.54
2	Group II	OSM Extract 100mg/kg	2.98±0.18	0.49±0.01	27.87±1.54	101.6±1.22
3	Group III	OSM Extract 200mg/kg	2.54±0.36	0.39±1.91	32.15±1.64	102.4±1.23
4	Group IV	CdCl2 50 mg/kg	7.7±1.91	0.98 ± 0.38	64.60±0.07	281±2.06
5	Group V	OSM 100mg/kg andCdCl2 50 mg/kg	4.4±1.54	0.78 ± 0.07	50.42±1.52	139.9±0.86
6	Group VI	OSM 200mg/kg and CdCl2 50 mg/kg	3.25±2.05	0.57 ± 0.02	37.40±0.97	131.24 ±0.54

Values are mean ± sem of 6 animals in each groups. Group IV compared with group I (P<0.001) Group II and III compared with Group IV (P<0.001). Group V and VI compared with IV (P<0.001) (ONE WAY ANOVA)

TABLE 2: EFFECT OF OSM EXTRACT ON BIOCHEMICAL PARAMETERS IN RATS SUBJECTED TO LEAD ACETATE INDUCED NEPHROTOXICITY

S.NO	GROUPS	TREATMENT AND DOSE	Blood Uric acid (mg/dl)	Serum Creatinine (mg/dl)	Serum Urea (mg/dl)	Urine Creatinine
1	Group I	Control	2.42±0.11	0.37 ±0.03	30±2.51	96.71±1.54
2	Group VII	Lead acetate 8mg/kg	6.54±1.34	0.93±0.05	59.96±1.64	199.2±0.86
3	Group VIII	OSM 100mg/kg and Lead Acetate 8mg/kg	3.52±0.91	0.47 ±0.04	38.20 ±0.10	141.24±0.67
4	Group IX	OSM 200mg/kg and Lead Acetate 8mg/kg	3.13±0.12	0.46±0.05	32.09±0.41	128.53 ±0.92

Values are mean ± sem of 6 animals in each groups. Group II compared with group I (P<0.001) Group III and IV compared with Group II (P<0.001). (ONE WAY ANOVA)

TABLE 3: REDUCED GLUTATHIONE (GSH) AND SOD LEVEL IN CONTROL, CdCl2 AND OSM TREATED RATS

S.NO	GROUPS	TREATMENT AND DOSE	GSH	SOD
1	Group I	Control	35.33±1.02	100.42±1.38
2	Group II	Cd Cl2 50mg/kg	17.66±1.32	53.31±1.96
3	Group III	OSM 100mg/kg and CdCl2 50mg/kg	30.12±2.22	62.29±1.78
4	Group IV	OSM 200mg/kg and CdCl2 50mg/kg	32.21±0.87	72.82 ±2.48

Values are mean ± sem of 6 animals in each groups. Group II compared with group I (P<0.001) Group III and IV compared with Group II (P<0.001). (ONE WAY ANOVA)

TABLE 4: REDUCED GLUTATHIONE (GSH) AND SOD LEVEL IN CONTROL, LEAD ACETATE AND OSM TREATED RATS

S.NO	GROUPS	TREATMENT AND DOSE	GSH	SOD
1	Group I	Control	35.33 ±1.02	100.42±1.38
2	Group II	Lead Acetate 8mg/kg	19.33±0.79	56.82±0.78
3	Group III	OSM 100mg/kg and Lead Acetate 8mg/kg	28.14 ±0.97	82.65±1.26
4	Group IV	OSM 200mg/kg and Lead Acetate 8mg/kg	33.33 ±1.12	95.14±1.07

Values are mean ± sem of 6 animals in each groups. Group II compared with group I (P<0.001) Group III and IV compared with Group II (P<0.001). (ONE WAY ANOVA)

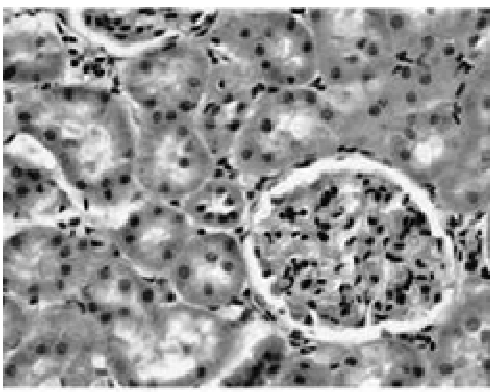

Figure 1: Histopathology of normal kidney section

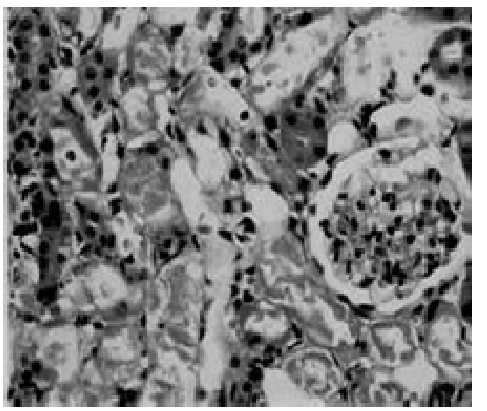

Figure 2: Histopathology of CdCl2 treated section

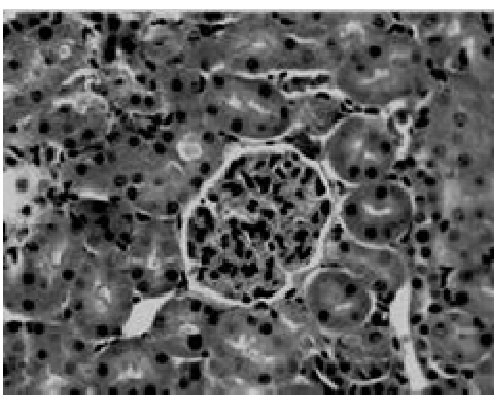

Figure 3 Histopathology of kidney treated with CdCl2 and 200 mg/kg OSM extract

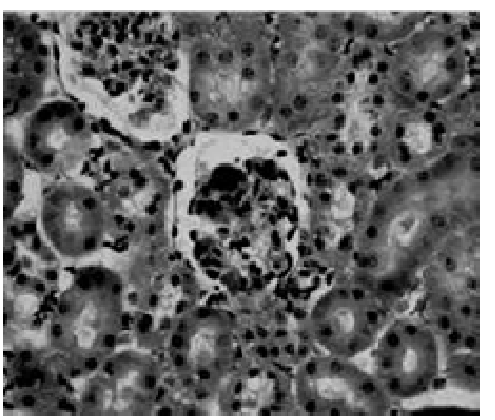

Figure 4 Histopathology of kidney treated with Lead Acetate and 200 mg/kg OSM extract

RESULTS AND DISCUSSION

The present study was conducted to evaluate the possible protective effect of *Orthosiphon Stamineus* leaves against the toxicological disorders induced by Cadmium Chloride and Lead Acetate in Male Wistar Rats particularly on Kidney. It is well known that heavy metals are widely distributed in environment and some of them can cause physiological, biochemical and histological disorders. Humans are exposed to these metals from numerous sources, including contaminated air, water, soil and food. Therefore, the evaluation of toxic potentials of metals is important for the risk assessment of human beings ordinarily exposed to these substances. The present work demonstrates that Rat chronically intoxicated with two heavy metals display a pronounced impairment in kidney function which is confirmed by the enhancement of plasma creatinine, urea and uric acid levels, and histopathological alterations. The results of the present work showed that the cortex is more affected than the medulla due to long-term treatment with heavy metals. This could be partly due to uneven distribution of heavy metals in the tissue of the kidney where about 90% of the total renal blood flow enters the cortex via the bloodstream. Accordingly, a relatively high concentration of these metals might reach the cortex via the bloodstream than that would enter the medulla.

Several studies demonstrated a significant enhancement of blood creatinine, urea and uric acid concentrations, and renal histological alterations in experimental animals intoxicated with Pb, Cd, and other heavy metals. From the present results, it is obvious that treating heavy metals-intoxicated rats with *Orthosiphon Stamineus* leaves significantly protected the kidney structure and function as compared to the controls. These observations were confirmed by insignificant alterations in the levels of plasma creatinine, urea and uric acid, kidney GSH and SOD, and an appearance of normal structures of kidney, specially renal corpuscles, *Orthosiphon Stamineus* Leaf Extract may be beneficial in reducing and slowing progressive kidney diseases that are significantly accelerated by oxidative stress. Additionally, demonstrated that the treatment with *Orthosiphon Stamineus* significantly reduced the changes caused by Cd and Lb exposure in all examined parameters. Moreover, suggested that these results indicate that alterations caused by Cd and Pb are connected with free radicals generation and used antioxidants effectively to protect against Cd and Pb intoxication.

In conclusion, the present study showed that *Orthosiphon Stamineus* leaves has protective effect on lead and cadmium induced renal toxicity. This study therefore suggests that *Orthosiphon Stamineus* leaves may be a useful preventive agent against the effect of the studied heavy metals.

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