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**Research Article** 

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# Anti-nephrotoxic perception of Diosmin, a citrus flavonoid inhibiting cadmium chloride induced oxidative stress in experimental rats

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## ABSTRACT

The aim of the present study was to examine the possible protective achieve of diosmin against Cadmium (Cd) induced renal toxicity. Male Wistar albino rats were divided into four groups of six animals each. Group II animals administered intraperitoneal (i.p) with CdCl<sub>2</sub> (2 mg/kg body weight/day) dissolved in saline for 10 days to tempt oxidative stress, alterations in urinary enzymes, lipid profile, and ATPase of nephrons in rats. Administration of CdCl<sub>2</sub> significantly induced renal toxicity which was evident from the increased levels of urinary constituents and altered biochemical enzymes. Cadmium Chloride stimulated kidney damaged rats were administrated with diosmin counteracts biochemical alterations were recovered. The swiftness of recuperation was more rapidly when diosmin (40 mg/kg body weight dissolved in saline once daily through oral administration for 28 days) was administered for management after the exposure of animals to cadmium. These findings suggest that diosmin may attenuate Cd induced renal toxicity and source for treatment of Cd mediated environmental disorder.

Keywords: Nephrotoxicity, Diosmin, Cadmium chloride, Kidney damage, Citrus flavonoids, CdCl<sub>2</sub>.

## INTRODUCTION

Nephrotoxicity due to drug or toxin is one of the most widespread kidney tribulations. Body unable to rid of excess urine and wastes from the body and blood electrolytes will all become elevated when kidney injury happen. Mixture of physiologic and biochemical proceedings donate to the propensity of the kidney to several distinct classes of nephrotoxicity [1]. When kidney is evaluated with other organs, the kidney is uniquely susceptible to chemical toxicity, partially because of its unduly high blood flow, and due to its intricacy both physiologically and anatomically [2]. Toxicity to nephrons may be consequent to direct cytotoxic damage to kidney structures by environmental toxicants, to immunologic processes, to indirect toxicity due to alterations in renal hemodynamics, or to the production of endogenous nephrotoxic substances [3]. As a result, the kidney concentrates toxicants in the filtrate, transports toxicants across the tubular cells, and bioactivates certain toxicants which attributes make kidneys extremely vulnerable to a variety of unfavorable outcome [4].

Cadmium (Cd) is a non-essential environmental toxicant has no known physiological occupation and it has been more widely used as an industrial product penetrate body through a variety of itinerary including food, water, air and by smoking of the cigarette [5]. Cadmium was primary acknowledged as a toxic element as the causative agent of Itai-Itai disease in Japan, a disorder mainly characterized by damage to the proximal renal tubule [6]. It enters the food chain, it poses a significant risk to humans and in blood it is mainly accumulated in the red cells and attach to a low molecular weight protein. Primarily Cadmium accumulates in liver and kidneys are found mainly as a complex bound with high affinity to detoxifying metal binding proteins, such as metallothioneins. It impinges on tubular epithelium resulting in increased cadmium in urine, aminoaciduria, glucosuria and decreased renal tubular reabsorption of phosphate [7]. Cadmium has also been demonstrated to inhibit many enzymes and competes with calcium metabolism and alters phosphorylation patterns [8]. A wide variety of cytotoxic and metabolic effects can be mediated by Cd, such as altering the activities of various enzymes, interfering with the normal protective actions of essential metals inducing oxidative stress, inhibiting mitochondrial ATP production, and altering gene expression [9]. Several studies have showed on the biological activities of flavonoids, the ameliorative effect of allicin on experimentally induced diabetes mellitus in albino rats that possess potent antioxidant effects [10].

Diosmin is a naturally occurring flavonoid glycoside that can be isolated from various plant sources or derived from the flavonoid hesperidin. The foremost intend of the present study to investigate cadmium induced kidney toxicity and nephroprotective ability of diosmin in cadmium intoxificated rats.

## **EXPERIMENTAL SECTION**

#### Chemicals

Diosmin was purchased from Sigma Chemical Company, USA. All other chemicals were of high purity analytical grade marketed by Sisco Research Laboratories Pvt. Ltd (SRL), India.

#### Animals

Healthy adult Wistar albino rats weighing  $160\pm20$ g were used in this study. They were obtained from the Central Animal House Facility, Dr.ALM PG IBMS, Taramani, University of Madras, Chennai, Tamil Nadu, India. The animals were kept in polypropylene cages and received standardized rat pellet and water *ad libitum*. The animals were kept in well ventilated room at a constant temperature with 12h day and night rhythm. All the procedures were done in compliance with the guidelines issued by the Institutional Animal Ethical Committee (IAEC No. 01/06/2014).

#### Experimental protocol

The adult Wistar albino rats were divided into four groups with six animals in each group and given dose regimen as given below.

Group I: Control animals given normal saline (0.9%).

Group II: Animals intraperitoneally (i.p) administered with  $CdCl_2$  (2 mg/kg body weight/day) dissolved in saline for 10 days. Group III: Animals exposed to  $CdCl_2$  were treated with Diosmin (40 mg/kg body weight) dissolved in saline once daily through oral administration for 28 days.

Group IV: Animals treated with Diosmin (40 mg/kg body Weight) dissolved in saline for 28 days orally.

#### Collection of urine samples

At the end of the experimental period the urine sample was collected on ice, which was free from fecal contamination. Urine samples were centrifuged and aliquots separated. One portion was acidified with concentrated HCl and used for analysis of urea, uric acid and serum creatinine. The remaining was dialyzed at 4°C against distilled water for 3 h and later was used for further biochemical analysis.

#### Collection of tissue sample

At the end of the experiment, animals were sacrificed under mild ether anesthesia by cervical dislocation. Blood samples were collected in tubes containing anticoagulant (EDTA) and tubes without EDTA. The serum was separated from whole blood after 30 min of coagulation at room temperature followed by centrifugation at 5000 rpm in a refrigerated centrifuge. Serum samples were stored at -20°C and serum biochemistry analysis was completed within a week after the initial storage. Both left and right kidneys were dissected out for biochemical, histopathological and electron microscopic analysis.

#### Biochemical analysis

 $\gamma$ -glutamyltranspeptidase ( $\gamma$ -GT) was determined [11], Lactate dehydrogenase (LDH) activity was estimated [12], Cathepsin-D activity was estimated [13], N-acetyl- $\beta$ -D glucosaminidase (NAG) activity was determined [14], Alkaline phosphatase and Acid phosphatase was estimated [15, 16],  $\beta$ -D-glucuronidase activity was estimated [17]

The inorganic phosphate was estimated [18], The free filtrate reacts erythrocyte membrane was isolated [19, 20], Na<sup>+</sup>/K<sup>+</sup>-ATPase was estimated [21], The activity of Ca<sup>2+</sup>-ATPase was assayed [22], The activity of Mg<sup>2+</sup> ATPase was assayed [23]. Total lipid was extracted and quantified [24], Cholesterol was estimated [25], Free cholesterol and free fatty acid was determined [26], Phospholipids were estimated [27], Triglycerides were estimated [28, 29].

#### Statistical Analysis

Data are presented as the mean  $\pm$  standard deviation (SD). One way analysis of variance (ANOVA) followed by Tukey's multiple comparison method was used to compare the means of different groups of by using SPSS 12.5 student's versions. Comparisons were made between group II and IV with group I and III for animal studies. p<0.05 was considerable statistically significant in all cases.

### RESULTS

Blood Urea Nitrogen (BUN), uric acid, total protein and creatinine levels in blood of control and experimental animals exhibited in table 1. Increased levels of (p<0.05) BUN, uric acid and serum creatinine were noted in the CdCl<sub>2</sub> toxicity group II animals. Administration of diosmin significantly decreased (p<0.05) in group III animals compared to that of group II animals. No extraordinary changes were noted in group IV drug alone treated animals when compared with group I control animals.

Table 1	1. Level	s of 1	urinary	constituents	s in b	lood o	of contro	l and	l experi	imental	anima	ls
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		Group II (CuCl <sub>2</sub> )	Group III (CaCl <sub>2</sub> + Diosmin)	Group IV (Diosmin)
Urea	21.37±1.74	38.81±0.24 <sup>a</sup>	30.62±1.01 <sup>a,b</sup>	21.81±0.07 <sup>b,c</sup>
Uric Acid	2.95±0.88	5.49±0.14 <sup>a</sup>	3.68±0.32 <sup>a,b</sup>	2.91±0.01 <sup>b,c</sup>
Creatinine	7.69±1.00	13.66±0.87 <sup>a</sup>	10.11±0.49 <sup>a,b</sup>	7.21±0.55 <sup>b,c</sup>
Total Protein	7.77±0.83	$16.45 \pm 2.76^{a}$	11.51±1.07 <sup>a,b</sup>	7.69±0.04 <sup>b,c</sup>

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 III Vs Group IV; The significance at the level of p < 0.05



Units: ALP, ACP and  $\beta$ -D-glucuronidase -  $\mu$  moles of p-nitrophenol liberated/mg protein/min,  $\gamma$ -GT – (IU/L), LDH -  $\mu$  moles of pyruvate liberated/mg protein/min, Cathespin D – n moles of tyrosine formed/hr/mg protein, N-acetyl - $\beta$ -D-glucosaminidase -  $\mu$  moles of phenol liberated/mg protein/min.

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 III Vs Group IV.

The significance at the level of p<0.05

Figure 1. Levels of urinary marker enzymes in urine of control and experimental animals

Figure 1 depicts the levels of marker enzymes in serum of control and experimental rats. The levels of  $\gamma$ -GT, LDH, Cathepsin-D, N-acetyl- $\beta$ -D-glucosaminidase, ALP, ACP,  $\beta$ -D-glucuronidase were increased (P<0.05) in toxicity induced group II animals when compared with group I control animals. Upon administration of diosmin all these enzymes level were notable declined (P<0.05) in group III animals when compared to group II toxicity animals. Drug control group IV animals did not show much variation when compared to control group I animals.

The effect of diosmin on lipid profile in serum and kidney of control and experimental animals are illustrated in table 2 and figure 2. In the present investigation, group II CdCl<sub>2</sub> increased nephrotoxicity rats showed significantly increased (p<0.05) in total cholesterol, free cholesterol, triglycerides, phospholipids and free fatty acids (p<0.05) when compared to that of group I control animals. These levels were significantly reverted to normal level (p<0.05) on treatment with diosmin when compared with group II. No remarkable changes were observed in group IV drug control animals.

Particulars (mg/dl)	Group I (Control)	Group II (CdCl <sub>2</sub> )	Group III (CdCl <sub>2</sub> +Diosmin)	Group IV (Diosmin)
Total cholesterol	69.86±2.84	107.14±0.55 <sup>a</sup>	86.37±0.23 <sup>a,b</sup>	69.77±1.28 <sup>b,c</sup>
Free cholesterol	27.65±1.89	42.33±3.01 <sup>a</sup>	34.22±0.09 <sup>a,b</sup>	26.88±0.79 <sup>b,c</sup>
Triglycerides	63.68±0.17	80.29±1.39 <sup>a</sup>	72.37±0.64 <sup>a,b</sup>	64.38±1.55 <sup>b,c</sup>
Free Fatty Acids	27.15±2.22	42.45±3.21 <sup>a</sup>	33.29±1.00 <sup>a,b</sup>	27.97±0.46 <sup>b,c</sup>
Phospholipids	38.13+0.66	57.14+0.67 <sup>a</sup>	$46.44 \pm 0.03^{a,b}$	38.71+0.83 <sup>b,c</sup>

Table 2. Levels of lipid profile in serum of control and experimental animals

Values are expressed as mean  $\pm$  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 III Vs Group IV; The significance at the level of p<0.05



Each bar 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 Figure 2. Display the levels of lipid profile in kidney of control and experimental animals

The levels of  $Na^+/K^+$ ,  $Ca^{2+}$  and  $Mg^{2+}$  ATPases in erythrocyte membrane and kidney of control and experimental animals were exhibited in table 3 and figure 3 respectively. Group II nephrotoxicity animals induced by cadmium chloride show a significant decrease in the levels of  $Na^+/K^+$ ,  $Mg^{2+}$  and  $Ca^{2+}$  ATPases (p<0.05) when compared with control group I. After diosmin treatment in group III animals the levels of membrane bound ATPase were found to be significantly increased (p<0.05) when compared to group II animals. No marked variations were found in group IV when compared with group I.

Particulars (mg/dl)	Group I (Control)	Group II (CdCl <sub>2</sub> )	Group III (CdCl <sub>2</sub> +Diosmin)	Group IV (Diosmin)		
Na <sup>+/</sup> K <sup>+</sup>	3.95±0.88	1.26±0.39 <sup>a</sup>	$2.24 \pm 0.76^{a,b}$	3.98±2.54 <sup>b,c</sup>		
Ca <sup>2+</sup>	4.44±0.50	7.38±0.11 <sup>a</sup>	5.13±2.07 <sup>a,b</sup>	4.72±3.83 <sup>b,c</sup>		
Mg <sup>2+</sup>	3.61±0.07	$1.19\pm0.48^{a}$	2.17±3.64 <sup>a,b</sup>	3.70±0.50 <sup>b,c</sup>		
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Table 3. Levels of ATPases in erythrocyte membrane of control and experimental animals

Values are expressed as mean  $\pm$  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 III Vs Group IV; The significance at the level of p < 0.05



Each bar expressed as mean  $\pm$  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 III Vs Group IV; The significance at the level of p < 0.05

#### DISSCUSSION

Renal injury due to Cd intoxication could be evaluated by appraising the serum and urinary markers of kidney damage which are the biochemical hallmarks of kidney destruction. Cadmium inoculation be evidence for severe renal damage associations with marked increase in the serum activity of urea and total protein, is mainly due to the leakage of these enzymes into the blood stream, which gives an indication of the renal toxicity [30]. In the present investigation increased urea, uric acid and total protein, decreased levels of creatinine clearance shows the diagnosis of renal damage in the cadmium treated rats. Renal markers of urea, uric acid, creatinine and creatinine clearance in serum, increased with the time of exposure to Cd and it is indicator represent biomarkers for renal damage [31]. Cadmium induced rats decrease in creatinine clearance that seems to reflect glomerular functional disturbances by Cd furthermore it induce mesangial glomerular cell contraction that was evidenced by a decrease in mesangial cell surface [32].These characteristic features of cadmium induced renal toxicity were similar to those previously reported by other toxicologists [33]. Management with flavonoid diosmin shields the nephrons and its normal occupation from cadmium induced free radicals in rats and restored the levels of those serum markers in the kidney. It may be possible, that diosmin, due to its potential antioxidant properties, metal chelating properties, improved renal function via attenuating oxidative stress- mediated decline in kidney and also proved its nephroprotective effects.

Urinary excretion of N-acetyl- $\beta$ -D-glucosaminidase (NAG), with a molecular weight of 120 kD, also has been reported to increase in humans exposed to cadmium [34]. This is a lysosomal enzyme present in high concentrations in the proximal tubule. Because of its high molecular weight, its urinary origin is unlikely to occur via glomerular sieving but rather directly from damaged kidney tissue. The levels of ACP in rats administered with cadmium chloride were elevated in every part of the damaged kidneys like cortex, medulla and papilla. The increased levels of ACP in the prostatic fraction and on the treatment with doismin produce a significant reduction in serum marker enzymes of ACP [35]. Administered with cadmium chloride showed elevated level of ALP and LDH were due to different types of kidney diseases. LDH increases in the renal cortical infarction may mimic pattern of acute

myocardial infarction [36]. It is also increased in renal infarction occasionally and decreased chronic renal dialysis. On treatment with diosmin produced a significant reduction in serum marker enzymes may be due to an improvement in the secretary mechanism of the renal tubules diosmin is to forage the free radicals and increases the levels of antioxidant properties.

Lipoproteins are complex particles consisting of lipids and different proteins. They are not passive containers of Triglycerides and cholesterol but dynamic particles with variable composition. Both lipoprotein turnover and composition is altered in kidney diseases [37]. Lipid abnormalities are frequently present in renal insufficiency and it has been considered as an important determinant of renal dysfunction [38]. It has been documented that Cd led to increased production of triglyceride and cholesterol [39]. Alterations in lipid profile and total cholesterol were observed in Cd administered animals. Exposure to Cd has been coupled lipoprotein abnormalities in plasma in experimental rats might be due to the mutilation of liver and kidney occupation caused by the disproportion in antioxidant defense in Cd intoxicated animals [40]. In the present analysis, the levels of total cholesterol and triglycerides in the serum were found to be elevated in cadmium chloride induced nephrotoxicity. The increased level of total cholesterol, triglycerides, phospholipids, fatty acids, free chlolestrol were observed in the serum and kidney of Cd induced animals. Diosmin has reverted back all the lipid contents to the normal level in serum and kidney tissue suggesting that diosmin play an essential responsibility in the management of lipid metabolism in kidney dysfunction.

ATPases maintains cellular electrolyte concentration and transmembrane electrolyte concentration and transmembrane electrochemical gradients. The Na<sup>+</sup> and K<sup>+</sup>-ATPases are ubiquitous membrane associated proteins expressed in most eukaryotic cells that maintains the high internal K<sup>+</sup> and low internal Na<sup>+</sup> concentrations typical of most animal cells. This electrochemical gradient provides energy for the transport of substrates and other ions across plasma membrane [41]. Peroxidation in tubular epithelium show the way to decrease in membrane fluidity and membrane integrity which delocalizes the enzyme Na<sup>+</sup>-K<sup>+</sup> ATPase from basolateral to apical membrane, while in glomerular filtration membrane diminish glomerular filtration rate. Thus both decreased glomerular filtration rate (GFR) and back leak of filtrate leads to imbalanced serum electrolytes [42]. Cadmium has high affinity for membranes of the sulfhydril group, which may account for the cell membrane disorganization. Cadmium inhibits  $Na^+/K^+$ - ATPase as well as  $Ca^{2+}$ -ATPase leading to an increase in intracellular calcium concentration. Calcium interacts with many heavy metals and may be an important factor in pathophysiologic mechanisms. The increase in intracellular calcium concentration caused by cadmium results from an increase in permeability in the protoplasmic membrane demonstrated in the  $Ca^{2+}$ -ATPase inhibition mediated by calcium efflux [43]. Mg<sup>2+</sup> ATPase activity is involved in other energy requiring process in the cell and its activity is sensitive to lipid peroxidation [44]. In the present investigation, a noteworthy dwindle activity of Na<sup>+</sup>/K<sup>+</sup>-ATPase and Mg<sup>2+</sup> ATPase and significant increased activity of  $Ca^{2+}$  ATPase in the heart were observed in Cd treated rats. Decreased activity of  $Na^+/K^+$ -ATPase could be due to enhanced lipid peroxidation by free radicals on Cd induction. Decreased activity of Na<sup>+</sup>/K<sup>+</sup>-ATPase can lead to a decrease in sodium efflux, thereby altering membrane permeability. Enhanced Ca<sup>2+</sup>-ATPase activity observed in Cd treated rats is due to the activation of adenylate cyclase by Cd. Calcium overload in the myocardial cells during ischemia activates the Ca<sup>2+</sup>-dependent ATPase of the membrane depleting high energy phosphate stores, thereby, indirectly inhibiting  $Na^+$  and  $K^+$  transport and inactivation of  $Na^+/K^+$ -ATPase. Elevated levels of  $Na^+/K^+$ concentration resulted in miserable effects of  $Ca^{2+}$  and augment  $Ca^{2+}$  influx and this could be due to the ability of diosmin to protect the "SH" groups from the oxidative damage through the inhibition of peroxidation of membrane lipids. In the present investigation, decreased levels of Na<sup>+</sup>, K<sup>+</sup> and Mg<sup>2+</sup> ATPase and cellular Ca<sup>2+</sup> overload in erythrocyte membrane and kidney, leads to substantial changes in cellular homeostasis seen in cadmium chloride toxicity. This result clearly indicates role of pathogenesis of the renal tubular cell injury produced by CdCl<sub>2</sub>. Diosmin management brought about a significant change in the activities of all these ATPase in the liver, erythrocyte membrane and kidney. Diosmin treatment modified the altered membrane fluidity and thereby improved the cell membrane integrity by modulating the activity of the membrane ATPases. In summary, our data demonstrated that organ toxicity and weaken kidney utility seen in cadmium chloride intoxicated rats were associated with oxidative damages in renal tissue and biochemical modifications. The study makes public that diosmin management protected against Cd induced renal dysfunction and oxidative stress.

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