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Remedial budding of Diosmin against Cadmium chloride induced experimental Nephrotoxicity

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ABSTRACT

Pharmacological potential of flavonoid diosmin beside oxidative damage of the organ. Generation of reactive oxygen species seems to play in important role in the pathogenesis of cadmium chloride induced acute renal failure. The present exploration was intended to demonstrate the efficacy of diosmin against cadmium chloride ($CdCl_2$) induced renal toxicity in animal model. Cadmium chloride ($CdCl_2$) arbitrated nephrotoxicity is remarkably renowned by reactive oxygen species and lipid peroxidation. Intra-peritoneal administration of $CdCl_2$ (2 mg/kg body weight/day) in group II animals put foundation of amplified lipid peroxidation, significantly increased renal oxidative stress, statically alteration in antioxidant defense machinery and smashed renal morphology. Flavonoids are low molecular weight and most abundant bioactive polyphenols which play a crucial responsibility in photosynthesizing cells. Animals exposed to $CdCl_2$ were treated with diosmin (40 mg/kg body weight) daily through oral administration for 28 days reduced toxic effect of toxicant in renal tissues, overturned $CdCl_2$ encouraged histopathological amendment and normalizes metabolic active biological enzymes. Therefore, present analyses accomplished that diosmin play down renal toxicity by blocking renal oxidative damage. Diosmin strongly proves its nephroprotective usefulness against $CdCl_2$ mediated renal oxidative damage.

Keywords: *Nephrotoxicity, Diosmin, $CdCl_2$, Cadmium Chloride, Renal toxicity.*

INTRODUCTION

Kidney is an extremely complex organ in terms of both anatomy and physiology. The primary function of the kidney is the excretion of waste products of the metabolism and plays a significant role in regulation of body homeostasis, regulating extracellular fluid volume and electrolyte balance. Thus the kidneys provide the final common pathway for the excretion of the drugs and their metabolites and xenobiotic chemicals [1]. Human beings are exposed to various kinds of xenobiotics as medicines, industrial and environmental chemicals and a variety of naturally occurring substances. The level of exposure may vary from minute quantities to very high dose. Primary feature of nephrotoxicity is impairment of normal functions of the kidney. Because of distinct anatomic and physiologic features, the kidney is uniquely susceptible to toxicity and is the target of many xenobiotics and environmental toxicants [2]. Nephrotoxicity may be consequent to direct cytotoxic damage to kidney structures by toxicants, to immunologic processes, to indirect toxicity due to alterations in renal hemodynamics or to the production of endogenous nephrotoxic substances. The clinical manifestations of toxic nephropathy vary from a mild reduction in renal function like hematuria, proteinuria and urolithiasis to a severe progressive toxicity resulting in end-stage renal disease [3]. Xenobiotic metals, such as aluminum, cadmium, lead and mercury are present in measurable concentrations in environment. Such metals often enter organisms by molecular mimicry, utilizing inherent transporters for essential metals. Environmental, occupational or intentional exposures to xenobiotic metals are frequently related to the development of toxicity and pathological conditions [4].

Cadmium (Cd^{2+}) is a non-essential toxic element which enters body via a number of routes including food, water, air and by smoking of the cigarette poses a significant risk to humans [5]. Cadmium is causing increasing concern as an environmental toxicant as it becomes more widely used as an industrial product and also accumulates in animals and plants, including tobacco leaves. Cd^{2+} mainly accumulated in the red cells and binds to a low molecular weight protein. More than 80% of cadmium is bound to metallothionein found in liver and kidney which plays a major role in homeostasis of cadmium. It is now widely thought that metallothionein is protective against cadmium toxicity and that intracellular cadmium bound to metallothionein is nontoxic [6]. It has been reported that pre-treatment of experimental animals with small doses of cadmium prevent acute toxic effects of large doses of cadmium. Acute cadmium poisoning may produce degenerative changes in renal tubular cells. In fact, studies indicate that up to 7% of the general population may develop renal dysfunction from Cd^{2+} exposure [7].

Flavonoids, with over 4,000 identified species, constitute a special class of polyphenolic compounds that are built upon a C6-C3-C6 flavone skeleton in which the two aromatic rings are linked by three carbons cyclized with oxygen [8]. Diosmin (3',5,7-trihydroxy-4'-methoxyflavone 7-rutinoside) is a naturally taking place flavonoid glycoside that can be isolated from various plant sources or derived from the flavonoid hesperidin [9]. Diosmin was first isolated in 1925 from *Scrophularia nodosa,* and first introduced as a therapeutic agent in 1969, considered to be a vascular-protecting agent used to treat chronic venous insufficiency, hemorrhoids, lymphedema, and varicose veins [10]. It also shows evidence of anti-inflammatory, free-radical scavenging, and antimutagenic properties has been investigated in a number of animal models and human cancer cell lines, and has been found to be chemopreventive and antiproliferative [11]. The foremost aim of the present study was to investigate the cadmium induced toxicity and to discover pharmacological ability of diosmin against cadmium induced renal pathology in experimental rats.

MATERIALS AND METHODS

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±20g were used in this study. They were obtained from the Central Animal House Facility, Dr.ALM PG IBMS, Taramani, University of Madras, Chennai-600113. The animals were kept in polypropylene cages and received standardized rat pellet and water *ad libitum*. The animals

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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. 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 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. 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 -20ºC 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.

Enzymatic analysis:

Tissue lipid peroxidation was measured [12], protein carbonyls were estimated [13], the activity of superoxide dismutase [14], catalase [15], and glutathione peroxidase were assayed [16], and the levels of glutathione [17], ascorbic acid [18], and vitamin E were estimated [19].

Histopathological examination

To investigate the histopathological changes and kidney tissue of various treatments, permanent mounts of the tissue samples were prepared. Kidneys of sacrificed animals were carefully dissected out for histopathological studies. After rinsing in normal saline, sections were taken from each harvested kidney. The tissue was fixed in 10% formal-saline, dehydrated with 100% ethanol solution and embedded in paraffin. It was then processed into 4-5m thick sections stained with hematoxylin-eosin and observed under a photomicroscope [20].

Statistical analysis:

Data are presented as the mean ± 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

Table 1 reveals the effect of diosmin on lipid peroxidation in the kidney of control and experimental animals. The levels of LPO were found to be significantly increase than in group II toxicity induced animals ($p<0.05$) when compared with control animals under basal conditions. Toxicity induced animals administrated with diosmin significantly diminish the peroxidation reaction in group III animals when compared with group II animals ($p<0.05$). However, no noteworthy revolutionize was observed in group IV drug control animals when compared to the group I control animals.

Table No. 1: Effect of diosmin on lipid peroxidation markers of kidney of control and experimental animals

Particulars	Group I (Control)	Group II (CdCl2)	Group III (CdCl2+Diosmin)	Group IV (Diosmin)
TBARS (M of MDA/g of protein)	17.18±0.25	49.11±2.34[a]	33.±0.58[a,b]	27.67±1.04[b,c]
Protein Carbonyls (nM/mg of protein)	5.88±1.33	10.54±0.89[a]	7.27±1.40[a,b]	5.79±0.02[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 2 exemplifies the activities of enzymic antioxidants and non-enzymic antioxidants in the kidney of control and experimental animals. Group II nephrotoxicity animals showed a significant minimize in the activities of enzymic and non-enzymic antioxidants ($p<0.05$) when compared with group I control. The levels of these antioxidants enzymes were augmented radically in diosmin treated group III animals when compared to that of group II animals ($p<0.05$). No astonishing amend were noted in group IV drug control animals.

Table 2 Effect of diosmin on Antioxidant status of kidney of control and experimental animals

Particulars	Group I (Control)	Group II ($CdCl_2$)	Group III ($CdCl_2$+Diosmin)	Group IV (Diosmin)
Super oxide Dismutase[#]	6.68±2.04	2.87±1.30[a]	4.41±0.55[a,b]	6.70±3.10[b,c]
Catalase	80.19±1.22	47.46±1.54[a]	62.22±1.90[a,b]	80.40±0.33[b,c]
Glutathione peroxidase[&]	4.78±0.55	1.60±0.89[a]	2.31±1.64[a,b]	4.79±3.01[b,c]
Reduced glutathione[^]	42.60±0.18	21±0.67[a]	35.11±0.28[a,b]	43.98±2.54[b,c]
Vitamin C[@]	8.90±1.56	3.47±0.49[a]	5.90±1.33[a,b]	8.88±2.07[b,c]
Vitamin E[@]	10.15±0.88	4.28±0.59[a]	7.35±1.62[a,b]	11.00±0.04[b,c]

[#] - units/mg protein; [&]-µ moles of H_2O_2 consumed/mg protein/min; [$]-µg of GSH utilised/mg protein/min; [^]- total thiol and Protein sulfhydryl - µg of GSH/mg protein; [@]- mg/g of wet tissue

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 $p<0.05$.

Histopathological studies on kidney of control and experimental animals are displayed in figure 1. The kidney of control rats showed no abnormality. Histological examination of kidney section showed marked and varying morphological alterations in cadmium chloride intoxicated animals comprise of vacuolation, tubular atrophy, severe tubular necrosis and interstitial inflammation. Diosmin treatment decrease the $CdCl_2$ induced tubular necrosis and most of the changes were caused by $CdCl_2$ intoxication and kidney displayed an almost normal architecture. Diosmin control group kidney showed normal architecture.

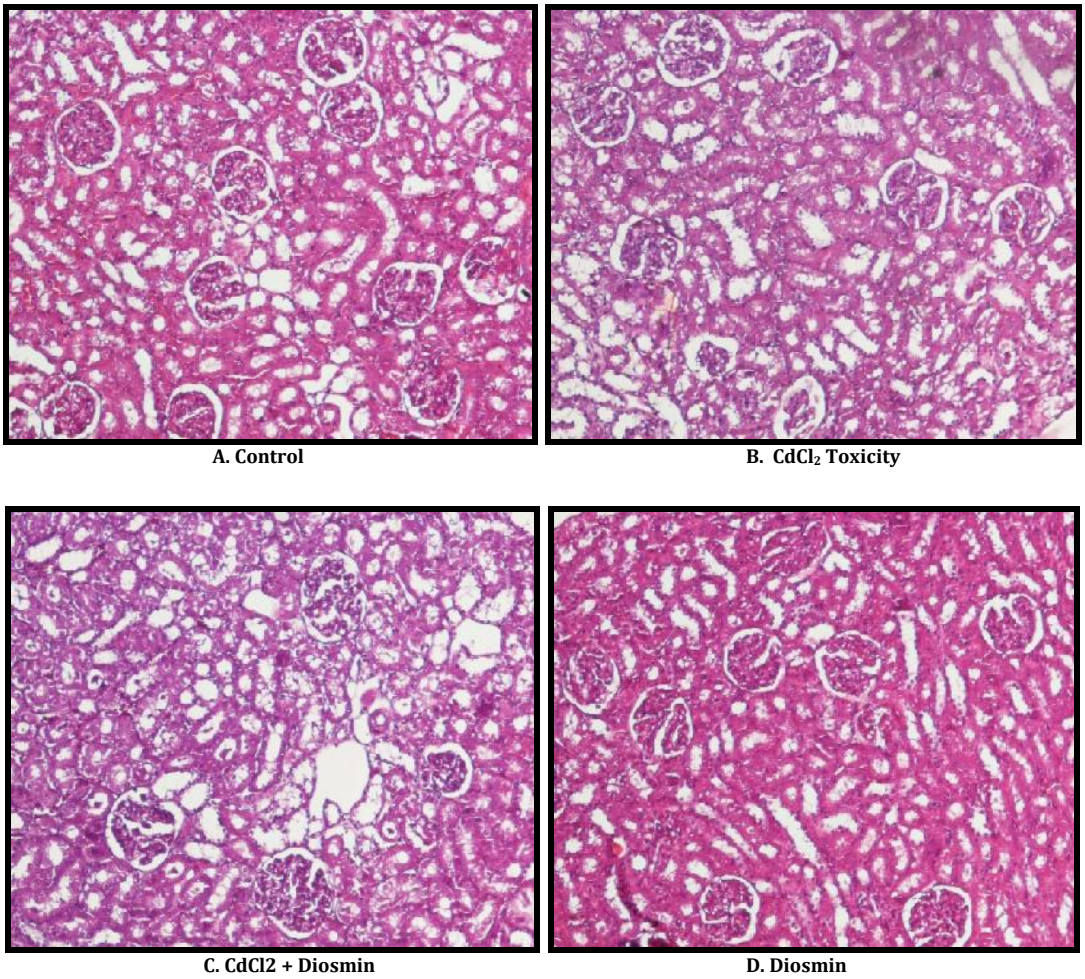

Fig. 1: Histopathological examination of kidney of control and experimental rats stained with Hematoxylin and Eosin
(**A**-Control animals show normal architecture; **B**-Kidney showed glomerular mesangial hypercellularity and proximal tubular necrosis; **C**-Renal tissue showing near to normal architecture; **D**-Showed normal architecture as control)

DISCUSSION

Lipids containing polyunsaturated fatty acids are susceptible to free radical-initiated oxidation and can participate in chain reactions that increase damage to biomolecules. Lipid peroxidation, which leads to lipid hydroperoxide formation often, occurs in response to oxidative stress [21]. Hydroperoxides are usually reduced to their corresponding alcohols by glutathione peroxidases. However, these enzymes are decreased in certain diseases resulting in a temporary increase of lipid hydroperoxides that favors their degradation into several compounds, including hydroxy-alkenals [22]. Cadmium is thought to induce lipid peroxidation and this has often been considered to be the main cause of its deleterious influence on membrane dependent function Most of the products of lipid peroxidation are unstable damage the membranes, cells and even tissues, latter is often used to assess oxidative stress [23]. Heavy metal accumulation and its toxicity effects are pronounced mainly in the kidney tissues to release more and more amount of LPO content was liberated. Because the release of Lipid peroxidation is mainly initiated by free radicals and is the oxidative deterioration of poly unsaturated fatty acids which is synthesized in intoxicated animals [24]. The significant increase in lipid peroxidation was found in kidney tissue of rat when treated with cadmium ions can act as potent pro-oxidant in kidney cortical cells, causing depolarization of mitochondrial inner membrane [25]. Cell membranes are phospholipids bilayers with extrinsic proteins and are the direct target of lipid peroxidation which leads to indirect production of lipid hydroperoxides and protein carbonyl content in the Cd treated rat kidneys. Early study reported that the cadmium induced kidney damage in rats was increased with the lipid peroxidation, lipid hydroperoxides and protein carbonyl content [26]. In the present analysis the increased levels of LPO may be interpreted as a result of the kidney cell destruction or changes in the membrane permeability. An enhanced LPO content are characteristic of kidney damage, therefore their release into the serum confirmed the CdCl2 induced kidney dysfunction. Management with diosmin strikingly disallowed renal oxidative stress induced by cadmium chloride in albino rats by reducing renal TBARS which undoubtedly exhibit anti-lipid peroxidative probable of diosmin.

Oxidative stress was caused by increasing oxidants and reducing antioxidants. Oxidants include reactive oxygen species (ROS) and reactive nitrogen species (RNS) which can be fashioned by both endogenous sources like inflammatory cells etc and exogenous sources such as cigarette smoke, exogenous toxins, pollution, radiation, carcinogens and drugs [27]. Under habitual physiological circumstances, oxidants are scavenged through antioxidant defense mechanism [28]. If moderately clearance by antioxidants, oxidants will be caused oxidative stress. Inefficiency and insufficiency of antioxidant defense system are concerned in some pathological conditions induced by oxidative stress [29]. Antioxidant enzymes are capable of stabilizing, or deactivating free radicals before they attack cellular components. They act by reducing the energy of the free radicals or by giving up some of their electrons for its use, thereby causing it to become stable. In addition, they may also interrupt with the oxidizing chain reaction to minimize the damage caused by free radicals [30].

Superoxide dismutase is metal-containing proteins that catalyze the removal of superoxide, generating water peroxide as a final product of the dismutation. Catalase (CAT) is heme-containing enzymes responsible for the degradation of hydrogen peroxide, and they are largely localized in subcellular organelles such as peroxisomes. CAT is a tetrameric protective enzyme present in nearly all animal cells. Glutathione peroxidase (GPx) is an enzyme that is responsible for protecting cells from damage due to free radicals like hydrogen and lipid peroxides [31]. Early investigation it was testimony that the levels of enzymatic antioxidant in cadmium treated rats kidney were significantly decreased due to the

production of ROS in the kidney [32]. In the present research depletion of enzymatic antioxidant levels in Cd treated rats may be increased oxidative stress due to the unpaired electrons in their atoms.

GSH (l-L-γ-glutamyl-l-cysteinyl glycine) is most copious tripeptide cellular antioxidant prevents the oxidation of protein thiol groups, either directly by reacting with reactive species or indirectly through glutathione transferases [33]. Vitamin C, and vitamin E, is most far and wide studied dietary antioxidants and vitamin C is measured the most important water soluble antioxidant in extracellular fluids. It is capable of neutralizing ROS in the aqueous phase before lipid peroxidation is commenced. Vitamin E, a major lipid-soluble antioxidant, is the most effective chain-breaking antioxidant within the cell membrane where it protects membrane fatty acids from lipid peroxidation [34]. Depletion of non enzymatic antioxidants in tissues leads to the impairment of cellular defense against ROS and may result in peroxidative tissue injury [35]. The decline non-enzymatic antioxidants in the present study prove that their absence may cause cell death due to free radical damage may be due to the accumulation of free radicals which in turn causes the inhibition of these enzymes. Thus in the present analysis cadmium chloride induced renal damage induced normally reflects disturbances and damages occurred in the kidney cells, which leads to not only characteristic changes of antioxidant enzyme activities but also inhibit its synthesis. In the present study, treatment with diosmin the levels of oxidant and antioxidant properties of kidney tissue was found to be near normal level. It may because of diosmin exert their antioxidant actions and defend the tissues from cadmium toxicants.

In the present investigation, histopathological studies of the kidney provided additional evidence that damaged renal cells recovered with diosmin treatment. The relative magnitude and degree of necrosis in proximal tubules based on quantitative histopathological analysis showed that the degree of renal damage observed was higher after hours of CdCl2 treatment [36]. In the present study, the renal histological structural design of the cadmium chloride intoxicated rats display various pathological modification in kidney. These alterations were exemplified by renal tubular damage, indicating by tubular necrosis. In our study, nephroprotective effects of diosmin were observed in glomeruli appeared to be restored tubules regeneration and less fatty infiltration. Toxicity induced kidney showed prominent recovery in the form of normal renal tubular and very less tubular necrosis upon treatment with diosmin. Control group had a regular histological structure and no histological variation was observed in the kidney of diosmin treated groups.

CONCLUSION

From the outcome of present research we concluded that diosmin due to therapeutic budding of renal damage, it holds back renal oxidative anxiety by blocking oxidative injury, upturned lipid peroxidation characterized by TBARS and restoration of the antioxidant enzyme status. Consequently diosmin restructure the morphological changes and noteworthy enhancement of renal function symbolizes a potential curative alternative to prevent cadmium chloride intoxicated oxidative renal tissue damage and dysfunction.

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