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Research Article

Histopathological Effects of Sub Acute Intoxication with Cadmium Administration on Kidneys and Heart of Rats

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Abstract

Histopathological effect of sub-acute intoxication of rats with cadmium (Cd) was studied in adult healthy male albino rats. Cd(cadmium chloride solution cdcl2 200mg/l) was administered in drinking water daily for 4 weeks. Nephropathy was achieved after4 weeks as indicated by biochemical assay. Microscopically examination showed various pathological alterations in kidney andheart of Cd treated rats. The results of this study indicated that sub-acute exposure of rats to Cd (200mg/l) in drinking water dailyfor 4 weeks induced alterations in blood biochemistry, renal and cardiac tissue structures.

Abbreviations:

MDA	:	Malondialdehyde
NO	:	Nitric Oxide
GOT	:	Glutamic OxaloaceticTransaminase
Na	:	Sodium
GPT	:	Glutamic PyruvicTransaminase
LDH	:	Lactate Dehydrogenase
NOH	:	Nitric Oxide in Heart
NOB	:	Nitric Oxide in Blood
NOK	:	Nitric Oxide in Kidney
TG	:	Tri Glycerides
LDL	:	Low Density Lipoprotein
HDL	:	High Density Lipoprotein
MDAH	:	Malondialdehyde in heart
MDAB	:	Malondialdehyde in Blood
MDAK	:	Malondialdehyde in Kidney
Hand E	:	Hematoxylin and Eosin
MNCs	:	Mononuclear Cells

Keywords: Biochemistry; Cadmium; Histopathology

Introduction

Nephropathy is a leading cause of morbidity and mortality and its prevalence is continuously increasing in industrialized nations [1]. The animal models for nephropathy share many features which are common to human nephropathy andhave been delineated by targeting proteinuria, glomerulosclerosis glomerulonephritis, glomerular hypertrophy, tubulointerstitialnephritis and tubular necrosis [1]. In this study, Cadmium (Cd) intoxication was designed as a model ofexperimental nephropathy.Cadmium (Cd) is a soft bluish-white metal that used largely in metal coatings, plastics and alloy batteries. Chronic environmentalexposure of Cd is nephrotoxic [2]**.** It has been noted that administration of Cd (0.18 mg/kg intraperitoneally,three times a week) for three months in rats led to hypertension followed by thickening of glomerular basement membrane,tubulointerstitial fibrosis and reduced glomerular filtration rate [3]. Administration of Cd (1 ml of 1 mM,intraperitoneally 3 times per week) for five, 20 and 40 weeks caused perturbation of kidney proximal tubular epithelial cells andmitochondrial dysfunction in renal cortical cells [4]. Cd may exert effects on the cardiovascular system atextremely low exposure levels. However, the exact influence of Cd on the cardiovascular system remains controversial [5]**.** The aim of this work was to evaluate the toxicopathological effects of subacute intoxication withcadmium administration on blood biochemistry and tissue structural alterations in kidneys and heart of rats.

Materials and Methods

Experimental animals

In this study, twenty adult healthy male albino rats with av-

erage weight 200-220 g were purchased from the animal house inHelwan and left for one week to acclimatize animal house in department of animal Physiology, Faculty of Veterinary Medicineand Mansoura University. Rats were kept under controlled environment, maintained under a 12 hours' light: dark cycle, 24°C (±3°C) and 50-70% humidity. All animal procedures followed the recommendations of the National Institutes of Health Guide forCare and Use of Laboratory Animals (Publication No. 85-23, revised 1985). All animal procedures were performed according tothe Ethics Committee of the National Research Centre, Egypt; registration number (09/189).5.2. Experimental designTwenty rats were randomly divided into 2 duplicate groups (five rats in each cage). The first group served as a control where ratswere provided with standard diet and water ad-libitum. Animals in the second group were subjected to freshly prepared Cdchloride solution cdcl2 (Sigma Company) 200mg/l in drinking water daily for 4 weeks; the whole duration of experiment [6].

Sample collection

After 4 weeks, blood samples were collected in plain test tubes via retro-orbital bleeding after 12 hours of fasting. Blood sampleswere left at room temperature for 1 hour then centrifuged for 10 minutes at 3000 g to obtain the serum. Serum samples werestored at-80°C for subsequent biochemical analysis. Five rats were killed each sacrifice by decapitation for collection of kidneysand heart. Each kidney was cut into two halves. After 4 weeks, one of kidney halves and rightventricles were obtained forestimation of Malondialdehyde (MDA) and Nitric Oxide (NO) levels in their tissue homogenates after been washed three times inice cold saline and blotted individually on ash-free filter paper. The crude tissue homogenate was centrifuged at 10,000 g for 15minutes in cold centrifuge, and the resultant supernatant was separated. Other halves of kidneys and left ventricles obtained fromboth sacrifices were fixed in 10% neutral buffered formalin until be routinely processed for histopathological examination.

Biochemical analysis

Urea and creatinine were measured in the serum by a colorimetric method using commercial kit (Diamon, Egypt) [7]**.**Serum lipid profile was estimated using commercial kits[8]. Glutamic Oxaloacetic Transaminase (GOT);Glutamic Pyruvic Transaminase (GPT), Lactate Dehydrogenase (LDH) activity was measured by a kinetic method usingcommercial kit (Egyptian company for biotechnology) [9]. MDA was determined spectrophotometrically [10]**.** NOB was assayed in the serum by a colorimetric method using the diazotization procedure according to Bartholomew(1984) [11], meanwhile, NOH and NOK were estimated in the heart and kidney homogenates as mentioned by Montgomery &Dymock (1961) [12].

Histopathological examinations

Paraffin sections from fixed kidneys and heart (5µm thickness) were cut and evaluated using standard staining protocol for H&E.Renal and cardiac slides from each group were also stained with Masson trichrome [13].

Statistical analysis

Statistical analysis of biochemical results was performed using the software SPSS 19 (SPSS Inc, Chicago, Illinois). Data wereexpressed as means standard errors. P values in the rows showed significance among groups after 4 weeks (P < 0.05).

Results

Biochemical assay

Nephropathy was noted by significant increase in levels of serum urea and creatinine, GPT, GOT, LDH, Na, NOH, NOB, NOK,cholesterol, TG, LDL, MDAH, MDAB and MDAK and significant decrease of HDL in Cd group (Table 1).

Measurements	Control	Cadmium	P value
Urea (mg/dl)	32.33±2.333^{a}	102.7±24.55^{b}	0.0006
Creatinine (mg/dl)	0.6700±0.03786^{a}	5.290±0.8110^{b}	0.0001
GPT (u/l)	15.33±0.8819^{a}	37.00±1.528^{b}	< 0.0001
GOT (u/l)	39.00±3.215^{a}	79.67±1.856^{b}	< 0.0001
LDH (ul)	259.0±8.386^{a}	368.3±19.22^{c}	< 0.0001
Na (mmol/l)	134.7±1.519^{a}	157.7±3.180^{b}	< 0.0001
NOH (umol/g)	24.30±0.4041^{a}	59.53±0.2603^{d}	< 0.0001
NOB (umol/dl)	19.20±0.4359^{a}	44.33±0.6692^{b}	< 0.0001
NOK (umol/g)	16.40±0.6245^{a}	40.10±0.2309^{b}	< 0.0001
Cholesterol (mg/dl)	110.0±5.033^{a}	215.3±22.00^{b}	0.0013
TG (mg/dl)	77.33±5.548^{a}	120.0±12.50^{ab}	0.0151
HDL (mg/dl)	49.00±5.568^{a}	23.67±0.8819^{b}	0.0003
LDL (mg/dl)	45.33±6.009^{a}	168.0±23.29^{b}	0.0009
MDAB (mmol/l)	24.83±0.3528^{a}	56.60±3.427^{b}	0.0004
MDAH (mmol/g)	31.90±0.5033^{a}	61.53±11.35^{b}	0.0146
MDAK (mmol/g)	24.37±2.534^{a}	66.30±5.805^{b}	0.0035
Means± SE Different superscript small letters in the same row indicate significant difference between groups when (P ≤ 0.05)			

Table 1: Biochemical measurements after 4 weeks.

Renal histopathology

Kidneys in the control group showed normal histological picture in both sacrifices. Meanwhile, kidneys of both sacrifices revealedthe presence of vacuolated and necrotic tubules with tu-

bular cast formation, collapsed and sclerotic glomeruli, fibrosis andmultiple foci of Mononuclear Cells (MNCs) aggregation in interstitial tissue (Figure 1& 2).

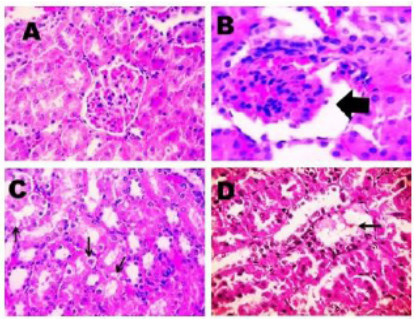

Figure 1 (A-D): Kidneys shows normal histological picture in control group (A), sclerotic glomeruli (thick arrow) (B), slightlyvacuolated tubules (thin arrows) (C) and focally necrotic tubule (D) (thin arrow) in Cd group (H&E A, C&D X: 100 and B, X:200).

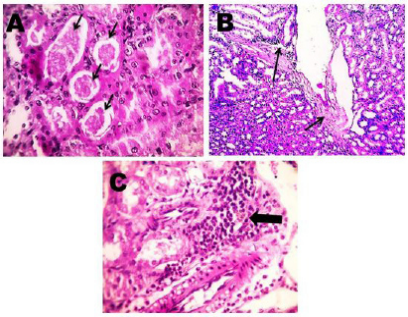

Figure 2 (A-C): Kidney of Cd treated group shows dilated renal tubules with hyaline casts (arrows) (A), focal area of fibrosis (shortarrow) with focal area of MNCs aggregation in interstitial tissue (long arrow) (B).(C) Higher magnification of (B) to show MNCsaggregation in interstitial tissue (thick arrow) (H& E A &C, X: 200 and B, X: 50).

Glomerular sclerosis and interstitial fibrosis were demonstrated in Masson trichrome stained slides (Figure 3)

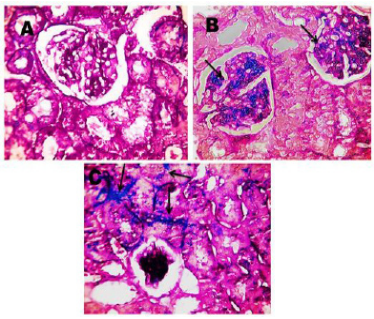

Figure 3 (A-C): Masson trichrome stained kidney slides shows absent glomerular and interstitial fibrosis in control rats, glomerular(B) and interstitial fibrosis (C) (arrows). in Cd exposed rats (A-C, X: 100).

Cardiac histopathology

Left ventricles in the control group showed normal histological picture in both sacrifices. Meanwhile, left ventricles in Cd treatedgroup revealed hyaline degeneration and mild per vascular fibrosis after 4 weeks. Hyaline degeneration with focal areas ofcoagulative necrosis and interstitial fibroblasts proliferation were observed in left ventricles after 8 weeks (Figure 4).

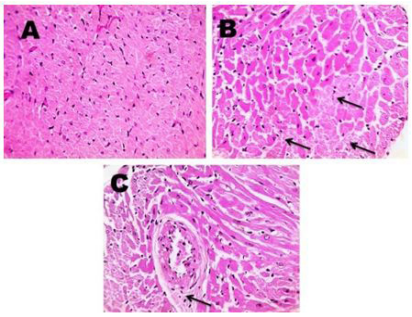

Figure 4 (A-C): Masson trichrome stained heart shows absence of fibrosis in control rats (A),per vascular fibrosis (arrow) in Cdexposed rats after 4 weeks (B) and interstitial fibrosis in Cd exposed rats after 8 weeks (C) (A&B, X: 100 and C, X: 200).

In Masson trichrome stained heart slides, interstitial fibroblasts proliferation was demonstrated (Figure 5).

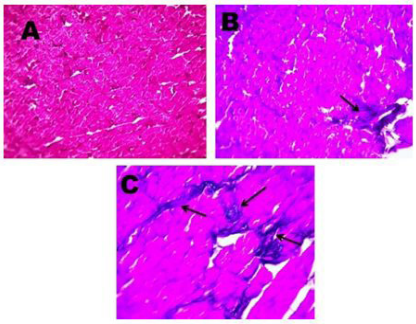

Figure 5 (A-C): Masson trichrome stained heart shows absence of fibrosis in control rats (A), pervascular fibrosis (arrow) in Cdexposed rats after 4 weeks (B) and interstitial fibrosis in Cd exposed rats after 8 weeks (C) (A&B, X: 100 and C, X: 200).

Discussion

Alterations in the blood biochemistry and tissue structures of kidneys and heart were achieved in Cd treated rats. Urea is the firstacute renal marker which increases when the kidney suffers any kind of injury; meanwhile, creatinine is the most trustable renalmarker and increase only when the majority of renal function is lost [14]. Renal injury was indicated after 4weeks by increased serum values of creatinine, urea, GPT, GOT, LDH, Na, NOH, NOB, NOK, cholesterol, TG, LDL, MDAH,MDAB, MDAK and decreased Level of HDL in Cd group in accordance with previous literatures [14-16]. Histopathological examination of kidney from Cd group demonstrated characteristic changes

Involving glomerular and tubular structures similar to those

previously mentioned by Aoyagi et al.(2003)[17]. The accumulation ofCd in the kidneys causes damage of the renal proximal tubules. The renal dysfunction induced by Cd has been considered one ofthe causes for the development of hypertension [18]. In addition, Cd induced alteration in heart tissue. The effectof Cd on the vascular system and cardiac function was previously discussed by Prozialeck et al. (2006)[19-24].

Furthermore, the correlation between blood and urine Cd concentration and diseases such as idiopathic dilated cardiomyopathy [25], Peripheral arterial disease [26], stroke, heart failure and atherosclerosis [27-29] were documented in many epidemiological studies.It was concluded that, Cd in drinking water (200mg/l) for 4 weeks induced alterations in blood biochemistry, renal and cardiac tissue structures.

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