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Evaluation of Toxicological Effects of Oral Administration of Cadmium Nitrate on Liver of Adult Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors SAA, ODO, UAY, O. O. Adeleye and O. O. Adeyinka designed the study and wrote the protocol. Authors SAA and ODO managed the animals, collected all data, performed the statistical analysis and wrote the first draft of the manuscript. Authors SAA, ODO and UAY did the literature search and also wrote part of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Available evidence suggest that adverse effect of health are caused by heavy metal and cadmium nitrate(CdN) has been shown to cause adverse health effect worldwide and liver is one of the organs mostly affected. The present study evaluates toxic effects of cadmium nitrate on liver of adult wistar rats.

Twenty (n=20) adult wistar rats of both sexes randomly divided into four groups (A-D) of five (n=5) rats each; Rats in group A served as control and were given 10 ml/kg/day of distilled water for 21 days. Rats in group B, C, and D (cadmium nitrate CdN group) were given 150 mg/kg/bw of CdN, 225 mg/kg/bw of CdN and 300 mg/kg/bw of CdN administered orally through an orogastric cannula

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into the stomach via the esophagus, once a day respectively, for twenty one (21) consecutive days. The gross anatomical parameters of the liver and liver histology were assessed. An assessment of the histological profiles of the liver showed distortions of the liver cytoarchitecture following consumption of CdN. Body weights of experimental rats decreased as compared to the control group. The mean liver weight also decreased as compared to the control. The serum levels of AST and ALT were significantly increased in CdN group compared to the control group. It was conclude that cadmium nitrate induced liver toxicity in adult wistar rats.

Keywords: Cadmium nitrate; liver; weight; heavy metal; wistar rat.

1. INTRODUCTION

Environmental pollution has many facets, and the resultant health risks include diseases in almost all organ systems [1,2]. Humans whose position in the food chain is at the top were exposed to various types of environmental contaminants at different stages of life, majority of which are harmful. In the environmental community, the notation of heavy metals implies stable high-density metals. These elements are natural constituents of the earth's crust. As a result of anthropogenic activity, the input of heavy metal to the environment has increased sufficiently and resulted in the increase of their content in air, water, soil and tissues of living organisms. Heavy metals are mostly toxic but their exact toxicity varies depending on the chemical form of the metal, point of exposure and the degree of exposure to the metal [2]. Metals when concentrated can be quite toxic and can result in death of organisms. Numerous hazardous heavy metals are inhaled and absorbed by humans and animals every day [3]. The major concern with heavy metals is their ability to accumulate in the environment and thereby passing up to food chain [4].

Cadmium (cd) is a vital component of modern technology, with many applications in the electronics, communication, power generation and aerospace industries [5]. Cd compound are commercially produced. Production and consumption of these compounds are increasing particularly in china, Korea, and Japan. The worldwide production of cd was approximately 17,200 tons in 2004 [6]. Once taken up by the blood, the majority of cadmium is transported bound to proteins, such as Albumin and Metallothionein [7].

The first organ reached after uptake into the GI-blood is the liver. Here cadmium induces the production of Metallothionein. After consecutive hepatocyte necrosis and apoptosis, Cd-Metallothionein complexes are washed into sinusoidal blood. From here, parts of the absorbed cadmium enter the entero-hepatical cycle via secretion into the biliary tract in form of Cadmium-Glutathione conjugates. Enzymatically degraded to cadmium-cysteine complexes in the biliary tree, cadmium re-enters the small intestines [8].

The main organ for long-term cadmium accumulation is the kidney [9]. Here the half-life period for cadmium is approx. 10 years. A life-long intake can therefore lead to a cadmium accumulation in the kidney, consequently resulting in tubulus cell necrosis.

The blood concentration of cadmium serves as a reliable indicator for a recent exposition; while the urinary concentration reflects past exposure, body burden and renal accumulation [10]. Excretion of Cadmium takes place via fasces and urine.

Cadmium nitrate occurs as a colorless solid. It is very soluble in dilute acids and soluble in ethanol, acetone, water, diethyl ether, and ethyl acetate [11]. Cadmium nitrate is available in technical and reagent grades with a purity of 99% or higher [12]. Systemic toxicity offer a means of understanding the mechanisms of free radical induced organ and or tissue damage. Among the tissues and organs in the mammalian body, liver and kidney seem to be the most sensitive predictor of chemical toxicity. This is due to their involvement in metabolism, detoxification, storage and excretion of xenobiotics and there metabolites, making them important target organs for xenobiotic induced injuries [13].

The present study was therefore designed to evaluate toxic effects of cadmium nitrate on histological damage of liver of adult wistar rats.

2. MATERIALS AND METHODS

2.1 Cadmium Nitrate

2.1.1 Procedure

The cadmium nitrate in this research was purchase from pharmaceutical shop in Yaba

Lagos, Lagos State, Nigeria in January 6,2015 and it was taken to Anatomy Laboratory of Department of Anatomy, College of Medicine, University of Lagos, Idi-Araba, Lagos, Lagos State, Nigeria and were authenticated by a staff in the Department of Chemistry University of Lagos, Lagos state Nigeria.

2.1.2 Animals and Diet

Twenty (n=20) adult wistar rats of both sexes were obtained from a breeding stock maintained in the animal house, the animal had free access to rat chow and tap water and they were randomly divided into four (4) groups (A-D) of five (n=5) rats each in a separate room at a constant temperature (22.0±1.0℃) under a 12 h light/dark cycle. Rats in group A which served as control were given 10 ml/kg/day of distilled water for 21 days. Rats in group B, C, and D (cadmium nitrate group) were given 150 mg/kg/bw cadmium nitrate, 225 mg/kg/bw of cadmium nitrate and 300 mg/kg/bw of cadmium nitrate administered orally through an orogastric cannula into the stomach via the esophagus, once a day respectively, for Twenty one (21) consecutive days. The average daily oral intake of cadmium by non-smokers living in unpolluted areas was estimated to 10–25 µg [14] while in laboratory animals oral LD50 values for mice and rats range from 60 to over 5000 mg/kg of body weight [15]. All experimental investigations were done in compliance with humane animal as stated in the "Guide to the care and use of Laboratory Animals Resources". National Research Council, DHHS, Pub.No NIH 86-23 [16] and in accordance with the guideline and approval of Nigeria Medical Ethical Association for Accreditation of Laboratory Animal Care.

2.2 Animal Sacrificed and Sample Extraction

Twelve hours after the administration of the last cadmium nitrate, the rats were at the time of sacrifice first weighed and then cervical dislocation was carried out following ethical humane animal euthanasia which was adopted with expertised cervical dislocation. The abdominal cavity of each rat was opened up through a midline abdominal incision to expose the liver. The liver was excised and weighed; the liver was weighed with an electronic analytical and precision balance. The liver of each animal was fixed in 10% formol-saline for histological examination. [BA 210S, d=0.0001- Sartoriusen GA, Goettingen, Germany].

2.3 Determination of Serum ALT, AST and ALP Assay

The degree of liver damage was evaluated by ALT, AST and ALP in serum using a commercially available kit. Detailed procedures for ALT, AST and ALP measurements were performed according to the kit manufacturer's instructions which was Sigma Aldrich products, made in Germany.

2.4 Histological Procedures and Analysis

This was done as described by Ogunlade et al. [17]. Briefly, the organs were cut on slabs about 0.5 cm thick and fixed in 10% formol saline for a day after which they were transferred to 70% alcohol for dehydration. The tissues were passed through 90% alcohol and chloroform for different durations before they were transferred into two changes of molten paraffin wax for 20 min each in an oven at 570 C. Serial sections of 5 µm thick were obtained from a solid block of tissue and were stained with haematoxylin and eosin stains, after which they were passed through a mixture of equal concentration of xylene and alcohol. Following clearance in xylene, the tissues were oven- dried. Photomicrographs were taken with a JVC colour video digital camera (JVC, China) mounted on an Olympus light microscope [Olympus UK Ltd, Essex, UK] to demonstrate the liver damage.

2.5 Statistical Analysis

All data were expressed as mean ± S.D. Differences between groups were analyzed using one-way analysis of variance [ANOVA]. A value of $p < 0.05$ was considered to be statistically significant.

3. RESULTS

3.1 Mean Body Weights, Liver Weights in Experimental and Control Rats

As showed in Table 2. Body weights of experimental rats decreased as compared to the control group. The mean liver weight also decreased as compared to the control.

3.2 Acute Oral Toxicity Studies

There was no death of rats at dose 400 mg/kg body weight of cadmium nitrate both within the

short and long outcome of the limit dose test of Up and Down method [Table 3]. The LD50 was calculated to be greater than 400 mg/kg body weight /orally.

Table 1. This table shows mean body weight and Standard Deviation of the experimental and control rats

Groups	Day 7 (Mean±S.D)	Day 14 (Mean±S.D)	Day 21 (Mean±S.D)
A(Control)	118±8.19	182±9.11	202±8.94
B	160±62.60	150±7.38*	156±3.57
C	138±5.39	162±3.34	178±5.90*
D	174±67.00	136±4.32	146±5.21*

*Value are expressed as n=5 mean±SEM *p<0.005*

Table 2. This table shows liver weight in experiment and control rats

Parameter	Group A(control)	Group B	Group C	Group D
Liver weight	1.21±0.29	1.12±0.01*	0.88±0.32	1.03±0. 30*

*Value are expressed as n=5 Mean±SEM *p<0.005*

Table 3. Results of acute toxicity test for cadmium nitrate

Test serial /No	Animal identity	Dose of cadmium nitrate mg/kg	Short term (72 hours)	Long term (12 days)
1	RET	400	+	+
2	LET	400	+	+
3	TC	400	+	+
4	RLT	400	+	+
5	I	400	+	+

+ -- Survival, REP—Right ear tagged, LEP—Left ear tagged, TP—Tail cut, RLT—Right leg tagged, I—Intact rat

3.3 AST and ALT levels

As showed in Table 4, the serum levels of AST and ALT were significantly increased in cadmium nitrate group compared to the control group following the exposure to cadmium nitrate.

Table 4. Shows AST and ALT levels in control and experiment rats

Groups	AST(U/l)	ALT(U/l)
A(Control)	26.0±0.1	24.3±0.2
B	110.9±0.4**	130.4±0.2**
C	110.2±0.1**	129.2±0.1
D	108.7±0.5**	127.2±0.1**

Values expressed as n=5 mean ± SD,
***p < 0.001 significant*

3.4 Liver Histology

Cross section of the livers of rats after the study showed Group A (control) had normal central vein (CV) with normal sinusoid (S) around the hepatocyte(H). [Fig. 1] Group B show mild steatosis, moderate interstitial inflammation and mild periportal inflammation. [Fig. 2] Group C show disseminated moderate macrovesicular steatosis and mild periportal inflammation. There is marked congestion of blood vessels and congestion of sinusoids with sinusoidal dilation. [Fig. 3].

Group D show mild steatosis and moderate congestion of blood vessels. There are moderate periportal inflammation. [Fig. 4].

3.5 Photomicrograpy Demostrations

3.5.1 Group A (control)

Photomicrographs of the liver of experimental animal administered 10ml/kg/day of distilled water as control.

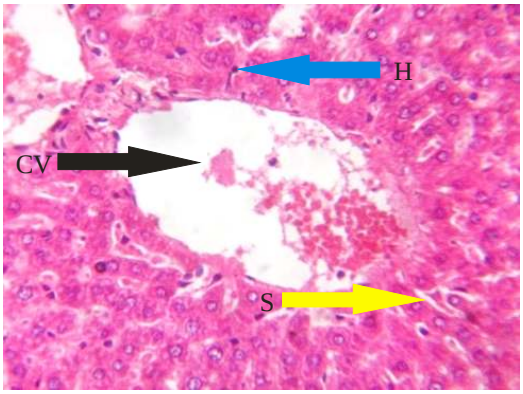

Fig. 1. Histological demonstration of the liver using H & E staining techniques [X400] showing normal central vein (CV, black arrow) with normal sinusoid (S, yellow arrow around the hepatocyte(H, blue arrow)

3.5.2 Group B

Photomicrographs of the liver of experimental animal administered 300 mg/kg/bw cadmium nitrate for twenty one days.

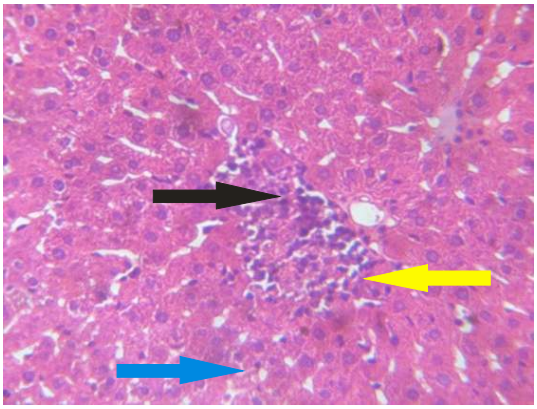

Fig. 2. Histological demonstration of the liver using H & E staining techniques [X400] showing mild steatosis (black arrow), moderate interstitial inflammation (blue arrow) and mild periportal inflammation (yellow arrow)

3.5.3 Group C (225)

Photomicrographs of the liver of experimental animal administered 225 mg/kg/bw cadmium nitrate for twenty one days.

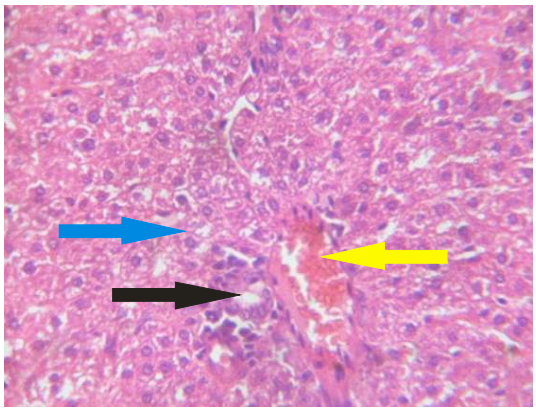

Fig. 3. Histological demonstration of the liver using H & E staining techniques [X400] showing disseminated moderate macrovesicular steatosis (blue arrow) and mild periportal inflammation (black arrow). There is marked congestion (yellow arrow) of blood vessels and congestion of sinusoids with sinusoidal dilation (black arrow)

3.5.4 Group D

Photomicrographs of the liver of experimental animal administered 150 mg/kg/bw cadmium nitrate for twenty one days.

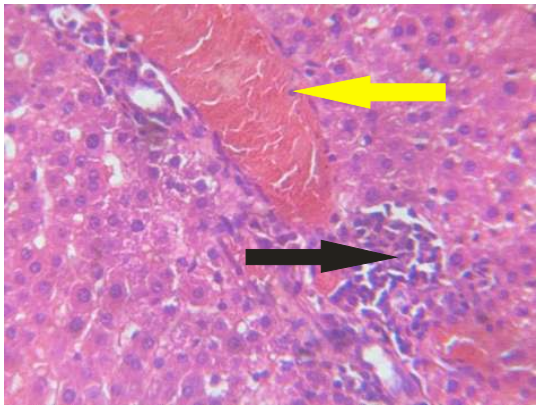

Fig. 4. Histological demonstration of the liver using H & E staining techniques [X400] showing mild steatosis and moderate congestion (yellow arrow) of blood vessels. There are moderate periportal inflammation (black arrow)

4. DISCUSSION

Water pollution has become a global problem. Heavy metals have long been recognized as serious pollutants of the aquatic environment. Health problems have been widely reported due to long-term ingestion of contaminated drinking water with heavy metals. Pollution of water bodies with heavy metals from variety of sources is becoming a matter of global concern [18]. Though effects of chemical contamination of drinking water are not felt on short-term basis (except nitrate), their accumulation over a long period in the body has significant health effects [19].

Present study demonstrates the toxic effect of administration of cadmium nitrate on the liver of experimental animals. Shows that exposure of liver to cadmium nitrate significantly increased serum intracellular enzymes such as aminotransferase (AST) and aspartate aminotransferase (ALT) compared to the control group. These are in accordance as reported by Tzirogiannis et al. [20] that AST and ALT levels increased for 10 hours after injection CdL. It was supported that AST and ALT was increased five hours after injection. m.AST is an indicator of mitochondrial injury and is important in the

clinical assessment of severe liver injury [21]. Osfor et al. [22] also reported that the levels of blood ALT, AST, ALP increased in Cd treated rats with several damages of liver tissues structure.

The histological evaluation can be used to shows the severity and toxicity of cadmium nitrate induced liver damage. It was observed in the control group that the normal hepatic cytoarchitecture was evident with visible terminal hepatic lobules consisting of terminal hepatic venules, hepatocyies with intervening sinusoidal spaces radially accentuated [Fig. 1]. Cadmium nitrate Group B show mild steatosis, moderate interstitial inflammation and mild periportal inflammation [Fig. 2]. Group C show disseminated moderate macrovesicular steatosis and mild periportal inflammation. There is marked congestion of blood vessels and congestion of sinusoids with sinusoidal dilation [Fig. 3]. Group D show mild steatosis and moderate congestion of blood vessels, there are moderate periportal inflammations [Fig. 4]. This is in line with the report of Osfor et al. [22] that administration of Cd in rats cause several liver tissue structure damages. We therefore deduced from our findings that oral administration of cadmium nitrate induced liver injury in adult wistar rats.

5. CONCLUSION

It can be therefore concluded from the present study that cadmium nitrate induced liver injury following the oral administration.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All the authors hereby declare that all the experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in line with the ethical procedure laid down by Nigeria Medical Ethical Association for Accreditation of Laboratory Animal Care.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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