*Scientia Africana, Vol. 13 (No.2), December 2014. Pp349-357*
 *ISSN 1118 - 1931*

# EFFECTS OF CADMIUM ION ON THE STRUCTURE AND FUNCTION OF RAT HEPATOCYTE

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*Received: 30-05-13*
*Accepted: 21-10-13*

## ABSTRACT

*The effect of cadmium ion on the structure and function of rat liver hepatocyte after oral (88mg/kg) and intramuscular (25mg/kg) administration of cadmium chloride for 14days was investigated by evaluating some liver marker enzymes, bilirubin and histopathological analysis. The result showed that both oral and intramuscular exposure to cadmium ion lead to significant increase ($p<0.001$) in the level of all the marker enzymes and conjugated bilirubin while total bilirubin showed no significant difference ($p<0.001$). The orally treated rats had alkaline phosphate (ALP) level of 99.40±0.28 IU/L aspartate transaminase (AST), 66.00±1.41 IU/L, alanine transaminase (ALT) 22.00±1.41 IU/L, total bilirubin (TB) 0.08±0.14mg/dl and conjugated bilirubin (CB) 0.70±0.14 mg/dl. Rats treated intramuscularly gave values of 114.00±2.83 IU/L for ALP, 116.00±4.24 IU/L AST, 36.99±1.41 ALT, 1.10±0.14 mg/dl TB and 1.00±0.14 mg/dl CB. Histopathological analysis showed a degeneration of hepathocytes and deposition of fatty materials. This result suggests that ingestion of cadmium ion has adverse effect on the structure and function of the liver.*

**Key words**: Cadmium, Hepato-toxicity, Histopathology

## INTRODUCITON

Cadmium is a relatively non-abundant element widely distributed in the biosphere. It is found in the earth's crust as part of several ores especially zinc-rich ores (Martelli et al, 2006). Cadmium makes up about 0.1ppm of the earths crust (Wedepohl, 1995). Naturally, a very large amount of cadmium is released into the environment into rivers through weathering of rocks and into the air through forest fires and volcanos (Trueman, 1965; Godt et al, 2006). It is also an inevitable by-product of zinc, lead and copper extraction (Ayres et al, 2010). After being released, it enters the environment mainly through the ground and appears in manures and pesticides. Cadmium is also released through human activities such as manufacturing (Morrow, 2010). Decades up to World War II, Japanese mining operators contaminated the Jinzu river with cadmium and traces of other toxic metals. This lead to cadmium accumulating in rice crops growing along the riverbanks downstream of the mine and consumption of the contaminated rice resulted in the development of itai-itai disease, renal abnormalities, protenuria and glucosuria (Nogawa et al, 2004). Cadmium is used in Ni-Cd batteries, pigments, electroplating of steel and plastic stabilizer. Phosphorous fertilizers contain cadmium as a

contaminant at levels varying from trace quantities to as high as 300mg Cd $Kg^{-1}$ of dry product and thus can be a significant source of cadmium input to agricultural systems (Grant and Sheppard, 2008, Taylor, 1997). Cadmium will accumulate in the soil treated with fertilizer if the quantity of cadmium in the fertilizer is greater than the amount removed during harvested crop removal and other loss pathways such as erosion, leaching or bioturbation (Grant and Sheppard, 2008). The major sources of airborne cadmium are smelters. Other channels of airborne cadmium include – burning fossil fuel such as oil and coal, burning of municipal refuse like plastics and nickel-cadmium batteries (Sahmoun et al, 2005).

Human uptake of cadmium takes place through foods. Fruits and vegetables particularly grains, potatoes and leafy vegetables cultivated in soils having high levels of cadmium are likely to contain elevated amounts of cadmium. Shellfish and organ meats such as liver and kidney also contain more cadmium than other food. Exposure of significantly high levels occur in smokers. Cigarette smoke is the major source of inhalative cadmium intoxication. The human lungs resorbes 40 – 60% of cadmium in tobacco smoke (Godt et al, 2006). The most dangerous form of occupational exposure to cadmium is inhalation of the fine dust and fumes or ingestion of highly soluble cadmium compounds (Morrow, 2010).

Although cadmium is found in the environment as part of several ores and has been used in many technological applications, biological systems generally have failed to safely deal with this element. Its only function in biology is as a replacement for zinc at the catalytic site of class of carbonic anhydrase in some marine diatoms (Maret and Moulis, 2013).

Mammalian exposure to cadmium compromises health and the mechanism of cadmium toxicity are numerous. This is because cadmium mimics other metals that are necessary for various biological activities. Metals prone to cadmium deceit include – calcium, zinc and iron. The inefficient cellular export system for cadmium explains the long residence time of the element in mammals (Martelli et al, 2006).

The toxicity of cadmium continues to be of significant public health concern since it enters the food chain and is taken up by tobacco smokers. Cadmium exposure is a risk factor associated with early atherosclerosis and hypertention which can lead to cardiovascular disease (Messner et al, 2009). It damages the kidney and lungs upon inhalation and meddles with bone metabolism. Youness et al (2012) reported that cadmium toxicity on bone via impaired activation of vitamin D is secondary to the kidney effect. Cadmium toxicity has also been mentioned as a possible source of male infertility in Nigeria (Akinloye et al, 2006). Cadmium is classified as a human carcinogen (Maret and Moulis, 2013). After ingestion, cadmium is transported to the liver where it stimulates the synthesis of metallothionein (Friberg, 1984). In this form, it is transported via the blood to the kidney where it accumulates and may damage the filtration mechanism leading to excretion of essential proteins and sugars from the body. Metallothionein plays a significant role in ameliorating cadmium toxicity resulting from prolonged exposure especially chronic cadmium induced

*Scientia Africana, Vol. 13 (No.2), December 2014. Pp349-357*
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nephrotoxicity, osteotoxicity and toxicity of the lung, liver and immune system (Klaassen et al, 2009).

Cadmium is also known to increase oxidative stress by being a catalyst in the formation of reactive oxygen species, increasing lipid peroxidation and depleting glutathione and protein-bound sulphydryl groups. Cadmium can also stimulate the production of inflammatory cytokinins and down regulate the protective function of nitric oxide formation (Navas-Acein et al, 2004).

Regardless of the route of exposure (oral, pulmonary or parenteral), the liver is to a large extent the primary organ that takes up the highest amount of cadmium during the initial periods after exposure. Some histopathological changes such as loss of normal architecture of the paranchymous tissue, cytoplasmic vacuolation, cellular degeneration and necrosis, fat globules etc were observed in liver tissue from rats exposed to cadmium for 22 days (Arroyo et al, 2012). The liver is necessary for survival and presently there is no anatomical or physiological compensation for its absence in the long term. Although cadmium accumulates in the liver and kidney, the chief organ of toxic impact in human is the kidney (Bernhoft, 2013). The effect of cadmium toxicity is becoming more prevalent due to industrialization, mechanized agriculture, illegal mining, mining and improper disposal of municipal waste. Further insight on the effect of cadmium accumulation in the liver on its structure and function necessitated this work.

## MATERIALS AND METHODS

**Animals:** Albino Wister rats having average weight of 200g were purchased from the University of Nigeria Teaching Hospital (UNTH) Enugu. The rats were kept in a well ventilated room and had free access to food (standard pellet feeds from Guinea Feeds Nig) and tap water. They were acclimatized for one week.

**Experimental Design:** Fifteen (15) albino rats of 200g average weight were divided into three (3) groups of five (5) rats per group. The first was administered $LD_{50}$: 25mg/Kg cadmium chloride dissolved in 1.0ml distilled water daily for fourteen (14) days intramuscularly. The second group had an oral administration of $LD_{50}$: 88mg/Kg body weight cadmium chloride in 10.0ml distilled water for the same number of days. The third group served as control. At the expiration of 14days, the rats were sacrificed under either anesthesia. Blood samples were collected via cardiac puncture and allowed to clot and serum was collected after centrifugation at 1000g for 10 minutes. The sera were kept at $4^0$C in a refrigerator prior to analysis of liver function. After exsanguination, the rat livers were excised, cleaned in normal saline and fixed in 10% formalin solution for histological examination.

### Histopathological Examination of Liver

The rat liver tissues were processed according to the standard paraffin procedure (Slaoui & Fiette, 2011). The fixed liver samples were dehydrated in graded alcohol and cleared in xylene before embedding in paraffin wax.

Sections of 4µm were cut using a microtome and staired in haematoxyline and eosin and then analyzed using the light microscope.

**Liver Marker Enzyme Analysis**

Aspartate and alkaline transaminase were analyzed spectrophotometrically using the methods described by Reitman and Frankel, (1957), while alkaline phosphatase was determined as described by Kind and King (1954).

**Bilirubin Determination**

Bilirubin was estimated using the method described by Powel (1944).

**Statistical Analysis**

Samples were analyzed individually in duplicates and the data were reported as mean±SD. The data obtained were subject to a one way independent measure ANOVA using SPSS version 16 and Microsoft Excel 2007.

## RESULTS

**Table 1: Effect of cadmium ion on some biochemical parameters; alamine amino transferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase (ALP) as indices of rat liver damage when administered 14 days intramuscularly (25mg/kg body weight) and orally (88mg/kg body weight) compared with the control.**

| | Intramuscular Administration 25mg/kg CdCl | Oral Administration 88mg/kg CdCl | Control |
|---|---|---|---|
| ALP | $114.00\pm2.83^{a}$ | $99.40\pm0.28^{b}$ | $78.10\pm1.13^{c}$ |
| AST | $116.00\pm4.24^{a}$ | $66.00\pm1.41^{b}$ | $12.00\pm1.41^{c}$ |
| ALT | $36.00\pm1.41^{a}$ | $22.00\pm1.41^{b}$ | $8.00\pm0.71^{c}$ |
| TB | $1.10\pm0.14^{a}$ | $0.80\pm0.14^{a}$ | $0.70\pm0.14^{a}$ |
| CB | $1.00\pm0.14^{a}$ | $0.70\pm0.14^{ab}$ | $0.34\pm0.03^{b}$ |

Values are mean± SD of duplicate results. Different superscripts within the same row indicates significant difference in the values ($p<0.001$) while same superscripts in the same row represents no significant difference ($p<0.001$). The values showed significant difference when compared with the control.

*Scientia Africana, Vol. 13 (No.2), December 2014. Pp349-357*
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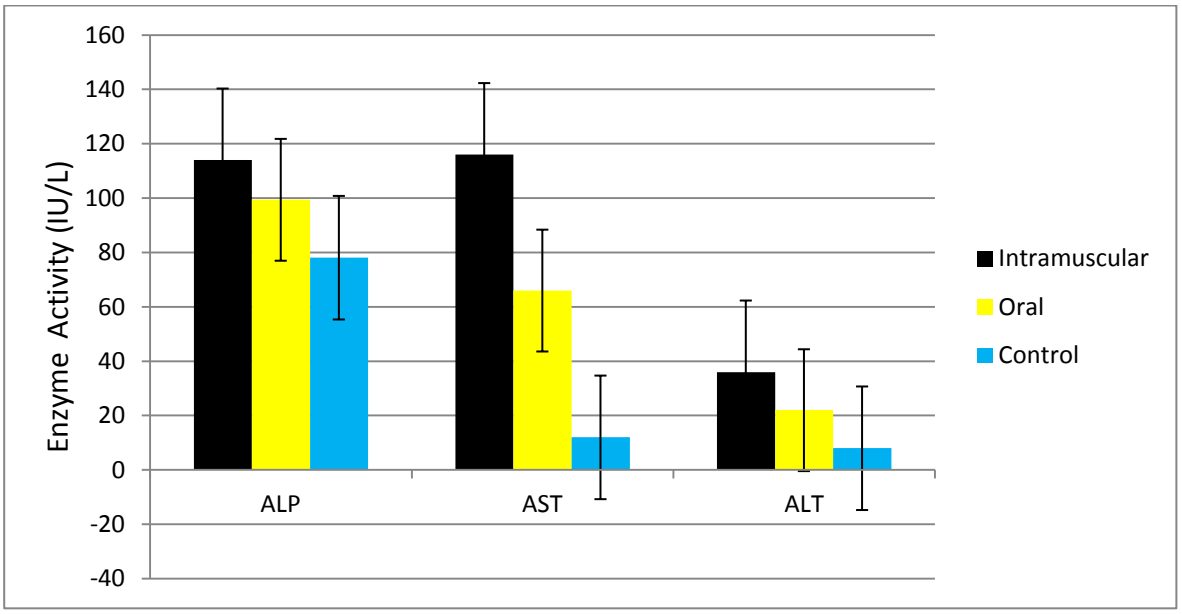



**Figure 1: A compound bar chart showing the mean±SD (activity) of alkaline phosphatase (ALP), alanine aminotransferase (ALT) and aspartate amino transferase (AST) in serum of albino rats exposed to oral and intramuscular administration of cadmium ion alongside the control. Activity is expressed as IU/L.**

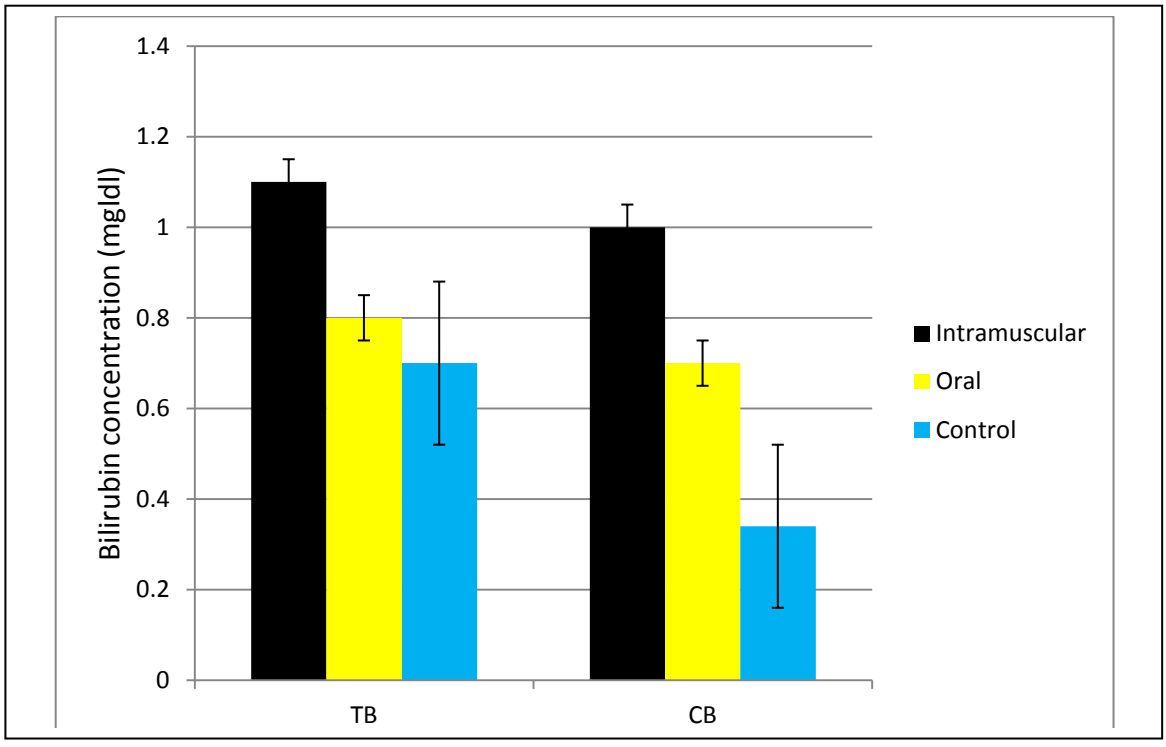



**Figure 2: A compound bar chart showing the mean±SD (concentration) of total bilirubin (TB) and conjugated bilirubin (CB) in the serum of albino rats exposed to cadmium ion administered orally and intrasmuscularly against the control. Bilirubin concentration is expressed as mg/dl.**

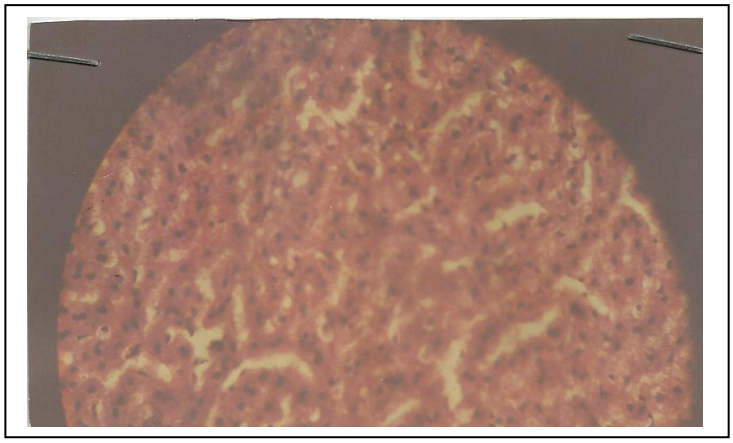
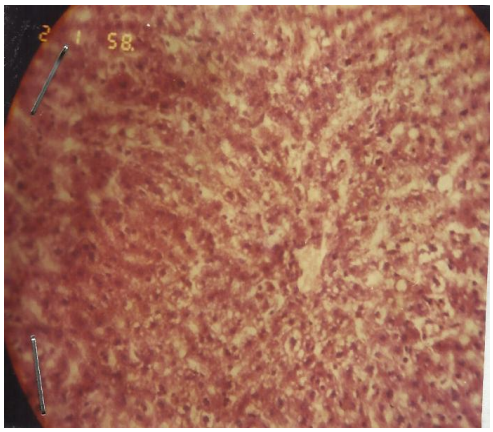


**Figure 3: Photomicrogram of the liver of rats showing normal hepatocytes inplates separated by sinusoids (H and E; X 400)**



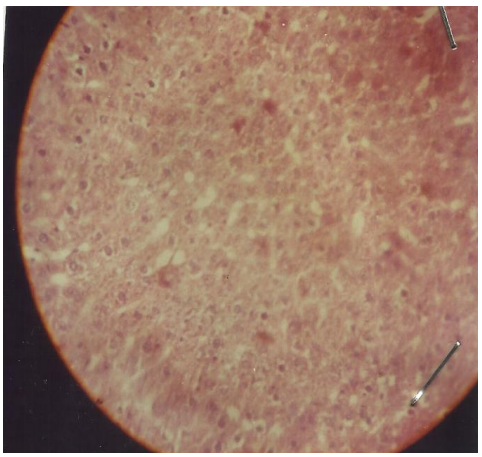
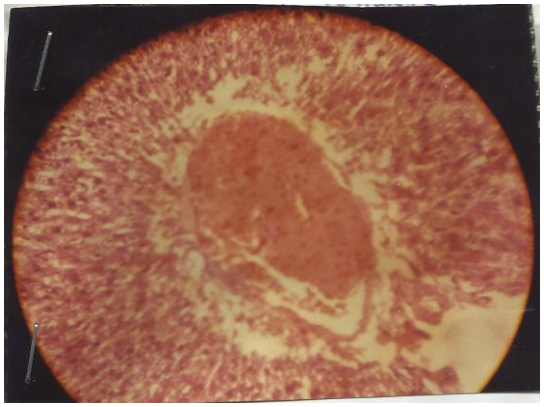


**Figure 4 & 5: Photomicrorgam of the liver of rats orally exposed to 88mg/kg CdCl showing mild hepatocyte degeneration and necrosis with the cytoplasm of some cells showing vacuolation (H and E; X400).**



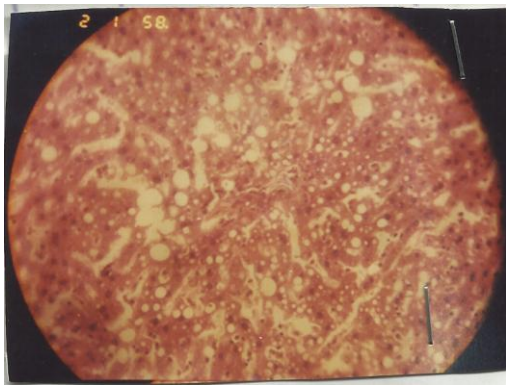


**Figure 6 & 7: Photomicrogram of the liver of rats exposed intramuscularly to 25mg/kg CdCl showing centrilobular and diffused hepatocyte degeneration with severe necrosis, congestion and multifocal pathological fatty infiltration (H & E; X400)**

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## DISCUSSION

The results of the biochemical evaluation of the effect of cadmium administration on the hepatocytes of albino rats revealed some undesirable effects. All the enzymes evaluated showed significant difference in their concentration ($p<0.001$) when compared with the control (Table 1). However, the values for the group exposed to cadmium ion intramuscularly was higher. The elevated values for alamine aminotransfrase (ALT) alkaline phosphatase and aspartate amino transferase (AST) points to possible injury of the hepatocytes by cadmium ion. These enzymes are usually present in intact hepatocytes and leak into the blood stream when liver cells are injured (Thapa and Walia, 2007).

Bilirubin estimation showed a significant difference in the level when compared with the control ($p<0.001$) for conjugated bilirubin, while there was no significant difference for the total bilirubin. The elevated level of conjugated bilirubin suggests a possible interference by cadmium ion with the capacity of the liver to carry out its transport and metabolic function. Bilirubin estimation is used to assess the capacity of the liver to transport organic anions and to metabolize drugs (Thapa and Walia, 2007). Furthermore, the increase in the level of bilirubin (the main bile pigment) suggests liver damage because elevation in serum bilirubin can be due to increase in destruction of red blood cells or decrease in the ability of the liver to remove it from the blood stream. Dudley et al (1998) and Arroyo et al (2012) reported severe liver injury in rats owing to acute exposure to cadmium. The present study appears to support these reports.

Histophathological analysis of the rat hepatocytes revealed mild degeneration and necrosis for the orally exposed rats, while the intramuscularly exposed rat hepatocytes showed severe necrosis, congestion, diffused hepatocyte degeneration and multifocal pathological fatty infiltration (Figure 3 – 7). The development of fatty infiltration in the liver is associated with the accumulation of fats in the liver cells. This can result from an increase in the amount of fat transferred to the hepatic gland from other part of the body or the reduced rate at which the liver breaks down and removes the fat. Any change in the steps involved in lipid metabolism can also lead to this disorder. It follows therefore that the administration of cadmium to these rats may have interfered with the ability of the hepatocytes to metabolize or clear the lipids leading to their accumulation and subsequent development of fatty infiltration. Cadmium toxicity has been established in several organs, the main organ of toxicity in men being the kidney (Bernhoft, 2013). The liver is a vital organ in animals. The finding in this work has shown the potential risk that may be associated with exposure to cadmium ion.

Globalization, industrialization, illegal mining, burning of fossil fuel incineration of municipal waste such as plastics and climate change appears to make the effects of cadmium toxicity more serious. As much as possible, man made exposure to cadmium should be avoided or minimized while methods of coping with exposure owing to natural disasters should be developed.

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*Scientia Africana, Vol. 13 (No.2), December 2014. Pp349-357*
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