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EFFECT OF ORAL CADMIUM ON HAEMATOLOGICAL INDICES AND LIVER FUNCTION OF ADULT MALE RATS: IMPLICATIONS FOR WARRI RIVER CADMIUM LEVEL.

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ABSTRACT

The effects of oraliy consumed cadmium on hematological indices and liver function have been investigated in rats based on the reported level of the toxicant In Warri River between 1986 and 1991. The results obtained after hematological analysis are indicative of cadmium induced aneamia in ras exposed to cadmium for three months only. Values obtained for control and test animals respectively were: $3.30 \pm 0.41 \times 10^6$ and $2.95 \pm 0.23 \times 10^6$ red blood cells/ml; 16.80 ± 0.76 and 15.83 ± 0.20 gHb/dL; 50.17 ± 1.15 and 45.17 ± 0.76 Hct. While no significant ($p<0.05$) change was observed in WBC counts in rats exposed to cadmium for one and three months relative to their respective controls, there was a significant ($p<0.05$) decrease in those exposed for two months: control – $6.40 \pm 2.90 \times 10^3$ WBC/ml and test – $4.30 \pm 1.30 \times 10^3$ WBC/ml. Biochemical analysis revealed a significant ($p<0.05$) increase in plasma L-aspartate aminotransferase (L-AST) activity in rats exposed to cadmium in all exposure periods relative to their respective controls. Values for plasma L-AST activity (units/ml) for the control and test groups in rats exposed for one, two and three months were respectively: 97.57 ± 3.39 and 115.17 ± 7.05; 183.92 ± 36.34 and 198.00 ± 19.56 and 216.00 ± 16.32 and 249.00 ± 46.02. The results for plasma L-AST suggest that the level of cadmium in Warri River waters during the period in question may be hepatotoxic.

KEY WORDS: Cadmium, Liver function, Hematology, Warri River.

INTRODUCTION

Alterations in haematological parameters and activity of serum enzymes are frequently indicators of toxicity and organ or cell damage (Kodavanti & Mehedale, 1991). Due to its toxicity, cadmium has been extensively studied in recent times. The metal has been reported to cause changes in blood parameters (Guilhermino *et al*, 1998). Increased levels of some serum enzymes [alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), sorbitol dehydrogenase (SDH)], total serum proteins and serum albumin were found in rodents treated with acute doses of cadmium (Theocharis *et al*, 1994; Funakoshi *et al*, 1995).

Drinking water is one of the major routes of human exposure to cadmium (WHO report, 1992) and maximum allowable level in drinking water is 0.005 mg/dL (WHO report, 1984). In many countries including Nigeria contamination of rivers and adjoining seas by cadmium and other heavy metals occur in a number of ways among which is the discharge of industrial wastes. Comprehensive and continuous monitoring of Warri River waters between 1986 and 1991 showed that the average level of cadmium was 0.3 mg/L (Egborge, 1994). This is 60-fold above the maximum allowable level in drinking water. Many communities along Warri River bank depend on the river as a source of drinking water. Consequently, people in these communities are at risk of suffering problems associated with cadmium toxicity. These problems include kidney, liver, bone and neurological diseases which are reportedly associated with cadmium toxicity (WHO report, 1992). This makes the presence of cadmium in Warri River worrisome. The purpose of this study therefore is to assess the likely health risk that may arise from exposure to simulated Warri River cadmium level using rat as an animal model. Specifically, the present study examined the effects of the level of cadmium reported in Warri River on the hematology and liver function of rats.

MATERIALS AND METHODS

Animals: Thirty six adult albino rats (Wistar strain), 180-190g, bred in the animal unit of the school of pharmacy, University of Benin were used for this study.

Chemicals and reagents: Cadmium sulphate, chloroform, sodium hydroxide, DL-aspartic acid, dinitrophenylhydrazine, alpha-ketoglutaric acid and sodium pyruvic acid were purchased from May & Baker, Dagenham, England.

Treatment of animals: The rats were divided into three groups of twelve rats each. Six of the rats in each group were given cadmium-free(deionised) water as drinking water and served as controls, while the other six were exposed *ad libitum* to aqueous solution of $CdSO_4$ containing the equivalent of 0.3 mg Cd/L which is amount present in Warri river (Egborge,1994). The first group of rats was treated for one month, the second for two months and the third for three months. All rats were

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Table 1. Initial and final mean body weight of rats used to study the toxicity of cadmium administered in drinking water.

Duration of Exposure (month)	Body Weight (g) Mean ± SD, n = 6			
	Control (-Cd)		Test (+Cd)	
	Initial Bd. Wt.	Final Bd. Wt.	Initial Bd. Wt.	Final Bd. Wt.
1	180.00 ± 6.80	218.20 ± 10.05 (21.22)*	186.00 ± 4.00	228.00 ± 12.30 (20.25)
2	193. 15 ± 5.60	254.75 ± 14.00 (31.91	185.92 ± 9.00	242.40 ± 16.50 (30.37)
3	209.27 ± 8.20	285.83 ± 16.80 (36.67)	201.42 ± 6.50	279.50 ± 21.60 (38.50)

*Figures in parenthesis represent percentage increase relative to initial body weight.
Bd. Wt. = Body weight.

Table 2. Haematological parameters of rats exposed to cadmium in drinking water (mean ± SD)

Duration of Exposure (month)	Haemoglobin Concentration (g/dL)		Haematocrit (Hct) (%)		White Blood Cell Count (WBC) $x10^3$		Red Blood Cell Count (RBC) $x10^6$	
	Control (-Cd)	Test (+Cd)	Control (-Cd)	Test (+Cd)	Control (-Cd)	Test (+Cd)	Control (-Cd)	Test (+Cd)
1	15.45 ± 1.32	15.78 ± 0.40	45.00 ± 4.00	46.00 ± 2.00	13.18 ± 2.50	14.13 ± 2.90	5.40 ± 0.70	5.60 ± 1.70
2	14.85 ± 0.45	15.18 ± 1.14	44.33 ± 1.03	45.00 ± 3.03	6.40 ± 2.90	4.30 ± 1.30*	4.92 ± 0.74	4.73 ± 1.43
3	16.08 ± 0.74	15.83 ± 0.20*	50.17 ± 1.15	45.17 ± 0.75*	9.10 ± 5.10	8.90 ± 4.80	3.30 ± 0.41	2.95 ± 0.23*

Mean ± SD, n = 6
*Values statistically significantly different ($p<0.05$) from controls.

allowed free access to chow (product of Bendel Feeds & Flour mills, BFFM ltd., Ewu, Edo state, Nigeria). The initial and final weights of each rat were also recorded.

Collection of samples: After the specified period of exposure, each rat was anesthetized with chloroform. While under anesthesia, blood was obtained from each rat via heart puncture and a portion transferred to heparinized tubes standing on ice. Plasma was obtained by centrifugation at 3000 rpm for 10 minutes and subsequently used for biochemical analysis. The other portion of whole blood from each rat was transferred to EDTA containers and used for haematological analysis.

Biochemical assay: Liver function of the rats was assessed by measuring the activities of aminotransferases in the plasma as described by Annino & Giese (1976).

Haematological analysis: Red blood cell (RBC) counts and White blood cell (WBC) counts were determined in blood using a cell coulter T_{540} (Coulter Electronic Ltd), based on the method described by Baker & Silverton (1978).

The packed cell volume (PCV) was also determined by the method of Baker & Silverton (1978). This involved the transfer of 0.8ml of blood into plain capillary tubes. The samples in the capillary tubes were centrifuged in the micro haematocrit centrifuge for 5 minutes at 5000 rpm, after which the percentage of blood PCV was calculated. The haemoglobin concentration of the blood sample was determined by the method of Fairbanks & George (1986).

Cadmium analysis: For cadmium analysis, 1g of liver was put into a beaker containing 20ml of acid mixture ($HNO_3/HClO_4$; 4:1 v/v) followed by heating at 100°C to facilitate digestion. The digests were allowed to cool and thereafter diluted with deionised water to give a final volume of 100ml. The cadmium concentrations in the digests were measured by atomic absorption spectrophotometry (Varian AA 1475 spectrophotometer). The test metal was also dissolved in deionised water and used as standard.

Statistical analysis: The data are presented as Mean ± SD. The mean values of the control and test groups were compared using student's t-test. The significant level was set at $p<0.05$.

RESULTS

The initial and final body weights of rats exposed to cadmium in drinking water are shown in Table 1. The results obtained show that there was no significant change in the mean body weight of rats administered cadmium relative to the control in all the exposure periods.

The results obtained for the haematological parameters are presented in Table 2. No significant ($p>0.05$) variation was observed in the hemoglobin concentration between the test and control groups in

Table 3: Activities of plasma alanine aminotransferase and aspartate aminotransferase activities in rats exposed to cadmium in drinking water.

Duration of exposure (Month)	Alanine aminotransferase (units/ml)		Aspartate aminotransferase (units/ml)	
	Control (-Cd)	Test (+Cd)	Control (-Cd)	Test (+Cd)
1	77.12 ± 3.11	79.71 ± 6.87	97.57 ± 3.39	115.17 ± 7.05*
2	117.00 ± 26.83	127.50 ± 39.69	183.92 ± 6.34	198.00 ± 19.56*
3	141.00 ± 32.67	131.00 ± 20.12	216.00 ± 16.32	249.00 ± 46.02*

Mean ± SD, n = 6
*Values statistically significantly different ($p<0.05$) from control.

rats orally exposed to cadmium for one and two months. However, there was a significant ($p<0.05$) decrease in haemoglobin concentration in those rats orally exposed to cadmium for three months relative to the controls. A similar trend was also observed in both the PCV and RBC counts. While no significant ($p>0.05$) change was observed in WBC counts in rats exposed to cadmium for one and three months there was a significant ($p<0.05$) decrease in those similarly exposed for two months.

Table 3 shows the changes of plasma aminotransferase activities of cadmium exposed rats. No significant variation was observed in L-alanine aminotransferase activity (L-ALT) in the plasma of rats exposed to cadmium for one, two and three months respectively, relative to controls. Conversely, there was a significant increase in the activity of L-aspartate aminotransferase (L-AST) in the plasma of rats similarly exposed relative to controls.

Table 4 represents the liver cadmium concentration of rats exposed to cadmium. The results obtained indicate a time dependent increase in liver cadmium concentration in rats exposed to cadmium in drinking water.

DISCUSSION

Exposure of rats to 100 and 200 ppm cadmium have been reported to decrease body weight (Zikic *et al*, 1998; Asagba *et al*, 2002). These doses are higher than the dose used in the present study. Thus the lack of significant ($p>0.05$) variation in the body weight of cadmium exposed rats relative to the controls (Table 1) may be related to the dose administered. Gastrointestinal absorption of cadmium is low (WHO report, 1992). Hence meaningful effects on body weight of rats may only occur after oral exposure to very high doses of cadmium.

The decrease in haemoglobin concentration, haematocrit and RBC counts of rats orally exposed to cadmium for three months (Table 2) is indicative of cadmium-induced anemia. Anemia has been reported as one of the most sensitive parameters of oral cadmium intoxication (Guilhermino *et al*, 1998). White blood cells (WBC) occur in large numbers in the blood. Since they are actively involved in the destruction of bacteria, they are therefore part of the body's immune system. Consequently, the significant decrease in WBC counts observed in rats orally exposed to cadmium for two months suggests that the immune system of the rats could have been compromised during this period of exposure to cadmium. Effects on the immune system have been reported after both acute and chronic cadmium exposures (WHO report, 1992). The effect of cadmium on the immune system has also been linked to increased susceptibility of rats to infection or other secondary dysfunction (Descotes, 1992).

Generally, the liver is one of the critical target organs after acute and chronic exposure to cadmium (Guilhermino *et al*, 1998; Kuester *et al*, 2002). Also, cadmium induced necrosis in the liver can cause the release of abnormal quantities of L-AST and L-ALT into the blood (Guilhermino *et al*, 1998). The increased plasma L-AST activity in rats of all exposure periods (Table 3) may therefore be an indication of liver damage. However, the lack of significant ($p>0.05$) variation in the plasma L-ALT activity in the test groups as compared to the controls (Table 3) is a likely indication that the damage to the liver (if any) was not severe enough to elicit an increased plasma activity of this enzyme. Though some animal experiments have shown that clinical tests generally used for studies of liver function may be negative even when morphological changes are manifest (Kamiyama *et al*, 1995).

Oral cadmium would rapidly be absorbed from the intestine and deposited in the liver and kidney (Elsenhans *et al*. 1997). A process that is aided by the induction of metallothionein, a cadmium binding protein. Thus the presence of cadmium in the drinking water may account for the significant increase of cadmium in the liver of the test rats (Table 4). The results presented in Table 4 for liver cadmium load also reveal that the

Table 4. Cadmium concentrations in liver of control and Cd-exposed rats.

Duration of Exposure (Month)	Concentration of Cadmium in Liver Mean ± SD; n = 6 $\mu g/g \times 10^{-2}$	
	Control (-Cd)	Test (+Cd)
1	2.50 ± 0.30	5.70 ± 0.70*
2	3.80 ± 0.20	9.30 ± 0.60*
3	3.60 ± 0.40	14.00 ± 5.00*

*Values statistically significantly different from control ($p<0.05$)

control rats were not cadmium-free. At the end of the first month of exposure the liver from the control rats had 0.025 µg Cd/g. By the end of the second and third months the liver cadmium had increased to 0.038 and 0.036 µg/g respectively. The presence of cadmium in the liver of the control rats may be an indication that their feed was tainted with cadmium. Similar results were obtained by other investigators (Tewari *et al*, 1986; Horiguchi *et al*, 1996; Crowe & Morgan, 1997). This is not surprising in view of the wide distribution of cadmium in the general environment of today. It is therefore possible that the result of our study is likely to be affected by the presence of cadmium in the liver of control rats. However, our test rats were provided with $CdSO_4$ solution prepared with the same deionised water that was provided as drinking water for the control rats. Both groups of rats were also maintained on the same feed. Evidently the control and the test rats likely had identical baseline cadmium level and effects or the same incremental build of cadmium from any other source upon which the load and effects due to $CdSO_4$ consumption will be superimposed.

In conclusion, the results obtained in this study suggest that in rats consumption of 0.3mg Cd/L the level of the metal in Warri River between 1986 and 1991 (Egborge, 1994) caused anemia in rats exposed for the maximum period of three months. There was also indication that this level of cadmium resulted in increased plasma L-AST of rats in all exposure periods. Therefore barring any species difference continuous human consumption of water contaminated to the extent reported by Egborge (1994) may likely cause anemia and some degree of liver dysfunction.

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