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Protective Effects of Vitamin C on Haematological Parameters in Intoxicated Wistar Rats with Cadmium, Mercury and Combined Cadmium and Mercury

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Abstract

Cadmium and mercury are reported as heavy metals that induce blood disorders and immunological effects. This study was performed to determine the haematological toxicity of cadmium, mercury and evaluated the protective antioxidant role of vitamin C. For this purpose, 65 rats were divided into 13 lots of 5 animals, grouped into 2 groups of 6 lots and one control group. Two different doses of each metal and their combination were administered orally for 28 consecutive days to 6 lots of 1 group. The first two lots (A, B) respectively were exposed to cadmium chloride, two other lots (C, D) were respectively received mercury chloride and the last two lots of this group (E, F) were respectively treated with the combination of these two metals. The second group of 6 lots (Ac, Bc, Cc, Dc, Ec, Fc) respectively have received over the previous doses of metals, a daily dose of Vitamin C during the same period. The control group (G) received the same volume of distilled water. At the end of exposure, the body weight of rats was weighed and whole blood was collected by retro-orbital sinus for analysis of haematological parameters. The results of this study showed a significant decrease ($P < 0.05$) on white blood cell, red blood cell, hemoglobin concentration, mean corpuscular, hemoglobin concentration with high concentrations of mercury and the combination of high concentrations of cadmium and mercury. However, co-administration of mercury, cadmium and mercury and vitamin C had a protective effect on the potential harmful metals.

Keywords: Cadmium, mercury, haematology, antioxidant, vitamin C, rat..

Introduction

The increase in the pollution of our day is a major and global problem. This is due to the use of toxic chemicals or xenobiotic substances or by certain synthetic compounds such as heavy metallic compounds[1]. Of these compounds, heavy metals, non-biodegradable induced potential effects at low doses. Discharged by industries[2], agriculture and urban communities[3], heavy metals reach the environment. Metallic compounds on land and water pose potential health hazard not only to livestock and wild life but also to fishes, birds, mammals and even to human beings. Also did not help in the metabolism, cadmium and mercury persist in the environment and are the most toxic metals to humans[4]. They have a strong ability to accumulate in the food chain, including halieutics products such as fish[5] and also in snails[6]. This situation exposes consumers to food poisoning. In acute, cadmium as well as in chronic cytotixicity and carcinogenesis, activation of endonucleases, generation of reactive free radical such as reactive oxygen species (ROS)[7,8] and signal transduction pathways involving apoptosis play important roles[9]. Cadmium accelerates lipid peroxidation by stimulating the peroxidation chain reaction in the target organs, resulting in the generation of ROS and consequently the induction of cytotoxicity[10]. Cadmium causes anemia[11,12] and induces immunological effects[13,14]. The classic symptoms of mercury contamination are carcinogenicity and/or damage to kidney function, visual, metabolic, reproductive, immunological and neurological[15-20]. Haematological and immunological effects induced by cadmium and mercury were verified by Guédénon *et al.*[21].

Studies have shown that vitamin C supplementation has varied effects on induced toxicity[22-28]. Ascorbic acid has been found to interact with several elements in such a way as to render them less available for animals[29]. He also demonstrated the protective effect of vitamin C on the toxicity of mercury[29]. Grosicki[30] and Akhere *et al.*[31] reported a decrease in the carcass cadmium burden and the cadmium contents in the liver, kidney, testicles and muscles of cadmium exposed rats given water supplemented vitamin C for 28 days.

Within this framework we proposed to explore the protective effect of vitamin C on blood disorders in rats exposed to cadmium, mercury and their combination.

Material and Methods

Biological Material: The animal material was composed of 65 male albino Wistar rats aged from 6 to 8 weeks and weighted about 108±25g. These rats obtained at the Animal Breeding Unit of the University of Lagos, Nigeria were acclimated for two weeks before the experiments. They were placed in designed sterile polypropylene cages in a room whose temperature borders 25-30°C with relative humidity of 60°C±5%. The cages were illuminated with a sequence of 12 hours with light and 12 hours into dark. Animals had free access to water and standard rodent laboratory chow (Ladokun feed Nigeria®) *ad libitum,* in the animal "Botanical and Zoological Garden" in UNILAG (University of Lagos), of Nigeria where experiments were conducted.

Chemicals and Preparation of the Various Solutions: The chemicals tests used for the experiment were cadmium chloride and chloride anhydrous of mercury. The powdered mercuric chloride ($HgCl_2$ = 271.50; minimum assay: 98%) and chloride cadmium ($CdCl_2$ = 183.32; minimum assay: 99%) were purchased from "General Purpose Reagent BDH Chemicals Ltd. Poolo England". Vitamin C (100 mg tablet), registered under MAFDAC REG. No. 04-1453, manufactured by 'Emzor Pharmaceutical Industries Ltd., Plot 3C Block A, was obtained "Outpatient Pharmacy Department of the Lagos University Teaching Hospital, Lagos, Nigeria" in Nigeria. The expiry date was scheduled for 02/01/2014. The vitamin C tablets not easily soluble were returned in the form of a fine powder prior to their solution was shaken vigorously before each oral administration. Concentrations were prepared for the experiment (0.25 mg/kg, 2.5 mg/kg) for cadmium chloride; (0.12 mg/kg, 1.2 mg/kg) for mercuric chloride and 150 mg/kg ascorbic acid. The different solution concentrations were based on different daily doses, the average weight of each lot and the daily volume administered to rats (1 mL).

Distribution of rats and testing: After two weeks of acclimatization, 65 rats were randomly divided into 13 groups of 5 pooled into 2 groups of 6 lots and one control group. Cadmium, mercury and their combination were administered by gavage (by stomach tube) for 28 consecutive days following the method of Alimba[32] and Awodele [22]. Six groups of rats received the first group daily doses of cadmium, mercury and their combination in a final volume of 1 ml of water. The first two batches (A, B) were exposed respectively to cadmium chloride (0.25 mg/kg, 2.5 mg/kg), two other batches (C, D) are respectively received mercury chloride (0.12 mg/kg, 1.2 mg/kg) and the last two batches of this group (E, F) were respectively treated with the combination of these two metals (0.25 mg/kg Cd + 0.12 mg/kg Hg) (2.5 mg/kg Cd +1.2 mg/kg Hg). The second group of 6 lots (A_C, B_C, C_C, D_C, E_C, F_C) have respectively received in addition to these previous doses of metals (Cd, Hg, Cd + Hg), a daily dose of 150 mg/kg vitamin C during the same period. The control group (G) received only the same volume of distilled water. These different doses of cadmium respectively correspond to a dose producing significant results: 0.25 mg/kg[33] and 10 times this concentration (2.5 mg/kg). As for mercury, 1/10 (1.2 mg/kg) and 1/100 (0.12 mg/kg) of the LD 50[34] were used.

Blood Collection and Haematological Analysis: After 28 days of exposure, rats were fasting overnight. They were weighed before the collection of blood and sacrifice and this to determine the final body weight. All samples were taken between 7 am and 9 am to avoid variations due to circadian rhythm. Whole blood was obtained from a puncture of the retro-orbital sinus by the conventional method[35]. This methodology is to slowly introduce the tip of the microhaematocrit (70 ml heparinized micro-capillary tubes haematocrit) in the medial angle of the eye. Progression through the tissue was facilitated by slightly turning to the pipette. The vessel wall was very fragile and when we reached the venous plexus, blood spurts and periorbital space rises by capillarity into the pipette that was put into tubes with anticoagulant for hematology. Blood samples collected in EDTA anticoagulant tubes (ethylene diamine tetra-acetic acid 8.5%) was quickly returned by mixing with anticoagulant in the tube. All blood samples were labeled and immediately conveyed to the laboratory for analysis. Haematological parameters were analyzed: white blood cell count (WBC), red blood cells (RBC), hemoglobin concentration (HGB), haematocrit (HCT), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT) and the number of lymphocytes (LYM). All haematological parameters were analyzed in the "Haematology Unit, Lagos state University Teaching Hospital (ULTH)" using the automated method with the automatic analyzer “Haematology auto analyzer Sysmex KX-21N”.

Statistical Study: Results are expressed as mean ± esm[1] of n experiments (where n represents the number of animals used). The differences between the treated and control rats were evaluated using the Students t-test p (T > t) = 0.05. The software used was Microsoft Excel 2010 and XL Stat 2011. The differences were statistically significant if the value of p was less than 0.05 and not significant if the value of p was greater than 0.05.

Results and Discussion

Haematological parameters analyzed were: Red Blood Cell ($X10^6$/µL RBC), White blood cell ($X10^3$/µL WBC), hemoglobin concentration (HGB) (g/dL HGB), haematocrit (% HCT), Mean Corpuscular Volume (fL MCV), Mean Corpusculaire Hemoglobin (pg MCH), Mean Corpuscular Hemoglobin Concentration (g/dL MCHC) Platelets ($X10^3$/µL PLT) and lymphocytes (% LYM). The results are presented as mean values and standard deviation of haematological parameters in the blood of rats exposed to cadmium, mercury and cadmium and mercury combination at various concentrations and intoxicated rats treated with vitamin C in table-1.

Table-1
Mean values and SD of hematological parameters of rats exposed to Cd, Hg and treated with vitamin C

Concentration	Parameters	control	Cd	Hg	Cd+Hg
		G	A	C	E
Low	WBC	13,95±0,21 a	13,55±1,76 a	10,17± 2, 32 a	8,47±1,50* b
	RBC	8,46±0,72 a	7,45±1,76 a	7,36±0,49 a	7,18±0,69 a
	HGB	14,40±0,35 a	13,60±2,40 a	13,47±1,57 a	13,32±1,12 a
	HCT	47, 450±0,07 a	44,95±8,83 a	46,47±5,20 a	45,07±4,84 a
	MCV	60,30±1,98 a	61,00±2,54 a	63,00±4,86 a	62,87±5,15 a
	MCH	18,20±0,42 a	18,50±1,13 a	18,25±1,40 a	18,60±1,10 a
	MCHC	31,30±0,84 a	30,30±0,56 a	29,00±1,07 a	29,60±0,89 a
	PLT	624,50±16,26 a	965,00±158,39 a	768,50±163,59 a	742,50±94,60 a
	LYM	69,60±10,46 a	69,00±10,46 a	69,87±11,96 a	69,80±6,29 a
		G	B	D	F
High	WBC	13,95±0,21 a	12,50±1, 20 a	9,85±0,49* b	8,15± 0,49* b
	RBC	8,46±0,72 a	7,78±0,66 a	5,59±1,02* b	5,46±0,78* b
	HGB	14,40±0, 35 a	14,47±0,96 a	10,37±1,97* b	9,65±0,77* b
	HCT	47,450±0,07 a	48,00±3,57 a	37,55±8,74 a	35,85±4,73 a
	MCV	60,30±1,98 a	61,00±1,91 a	66,62±5,86 a	60,50±0,72 a
	MCH	18,20±0,42 a	18,60±0,58 a	18,52±0,48 a	18,45±0,77
	MCHC	31,30±0,84 a	30,20±0,29 a	27,35±1,39* b	27,00±0,70* b
	PLT	624,50±16,26 a	901,50±241,41 a	822,50±378,70 a	841,00±427,09 a
	LYM	69,60±10,46 a	71,32±11,02 a	65,75±5,47 a	65,30± 4,38 a
Concentration	**Parameters**	**control**	**Cd + Vit C**	**Hg + Vit C**	**Cd+Hg+ Vit C**
		G	Ac	Cc	Ec
Low	WBC	13,95±0,21 a	24,40±5, 39 a	13, 95± 2, 76 a	14,05±5,20 a
	RBC	8,46±0,72 a	7,72±1,07 a	7,49± 0,30 a	7,305±0,78 a
	HGB	14,40±0,35 a	13,55±1,52 a	13,32±0,44 a	12,72±0,69 a
	HCT	47, 450±0,07 a	47,50±6,02 a	43,67±0,80 a	43, 95±3,65 a
	MCV	60,30±1,98 a	61,70±3,22 a	58,32±1,99 a	69,30±2,13 a
	MCH	18,20±0,42 a	17,60±0,67 a	17,80±0,45 a	17,50±1,27 a
	MCHC	31,30±0,84 a	28,57±1,49 a	30,50±0,78 a	29,02±1,38 a
	PLT	624,50±16,26 a	1078,75±180,28 a	874,75±46,42 a	1254,50±262,67 a
	LYM	69,60±10,46 a	54,82±14,15 a	55,32±13,98 a	52,30±15,39 a
		G	Bc	Dc	Fc
High	WBC	13,95±0,21 a	14,75±1,93 a	14,70±4,18 a	10,70±2, 34 a
	RBC	8,46±0,72 a	7,45±1,26 a	8,10±0,70 a	8,02±0,12 a
	HGB	14,40±0, 35 a	14,17±0,35 a	14,93±0,68 a	14,25±0,45 a
	HCT	47,450±0,07 a	46,12±7,58 a	47,00±3,12 a	46,30±1,49 a
	MCV	60,30±1,98 a	61,50±1,05 a	58,03±1,30 a	60,30±2,13 a
	MCH	18,20±0,42 a	21,22±5,27 a	18,46±0,65 a	17,77±0,34 a
	MCHC	31,30±0,84 a	30,85±1,27 a	31,80±0,72 a	30,77±0,56 a
	PLT	624,50±16,26 a	734,00±248,17 a	1014,33±110,18* b	907,25±120,40* b
	LYM	69,60±10,46 a	49,35±7,86 a	60,86±14,23 a	57,75±9,24 a

***** The difference between the value of blood parameters of the experiment groups and that of the negative control at 0, 05. The averages followed by the same letter are not statistically different; a and b (significativity enters negative control, lots intoxicated at metals and their correspondent intoxicated and treated with vitamin C; a: not significant and b: significant)

Interpretation of Results: Count of Red Blood Cell: In Wistar rats, erythrocyte count has made important and significant variations ranging from 8.46±0.72 in animals of Lot G (control) to 5.59±1.02 and 5.46±0.78 in those of Lot D and F ($p < 0.05$). The red blood cell count was significantly higher (8.10±0.70 and 8.02±0.12) in rats and lots Fc Dc when compared to that of rats of Lot D and F but lower than that of rats of Lot G ($p < 0.05$).

Count of white blood cells: The lowest values of 9.85±0.49, 8.47±1.5, 8.15±0.49 for leukocytes was encountered respectively in rats lots D, E, F against 14.70±4.18, 14.05±5.20 and 10.70±2,34, 13.95±0.21 for animals in Dc, Ec, Fc and G ($p <0.05$). As for platelets, the number has increased in all rats intoxicated (A, B, C, D, E, F) compared to control (G) and more increase with the application of vitamin C (Ac, Bc, Cc, Dc, Ec, Fc).

Hemoglobin: Hemoglobin concentration was significantly lower in rats of group D and E (10.37±1.97 and 9.65±0.77) respectively when compared to (14.40±0.35, 14.93±0.68, 14.25±0.45) than rats of lots G, Dc and Fc ($p <0.05$).

Haematocrit : The haematocrit values in Wistar rats ranged from 47.45±0.07 in animals in the G (37.55±8.74 and 35.85±4.73), respectively, in those of group D and F through intermediate values (47.00±3.12 and 46.30±1.49) rats lots Dc and Fc.

Mean corpuscular hemoglobin concentration: The average concentrations corpuscular hemoglobin concentration (MCHC) were fluctuating values with the lowest (27.35±1.39 and 27.00±0.70) respectively in the rats of groups D and F compared to those of other groups ($p < 0.05$).

The results of this study found that some parameters vary widely depending on the batches of Wistar rats intoxicated with cadmium, mercury and their combination and treated with vitamin C. This is for example the case of the erythrocyte: indeed animals of lots D and F, it reached values $5.59.10^6$/uL and $5.46.10^6$/µL against $8.46.10^6$/uL animals of Lot G (control) with intermediate values in animals treated with Fc, Dc and vitamin C. The number of red blood cells obtained from animals in the D and F is very low compared to the results of Boukerche *et al.*[36], where these authors found $8.45.10^6$/uL as the number of red blood cells in healthy Wistar rats. It is likely that the high dose of mercury and the combination of high doses of cadmium and mercury do reduce the number of red blood cells causing anemia in Wistar rats intoxicated. These results have been shown by other authors[37,38] conducted on mice poisoned with lead. These authors argue that the number of red blood cells decreased. The red blood cell count not intoxicated rats (control) is stable and varies little from one subject to another. Even if this stability seems characteristic of healthy Wistar rats, the number of red blood cells obtained from rats of Lot G in this study is almost similar to $8.45.10^6$/uL obtained by Boukerche *et al.*[36] but higher value $7.1.10^6$/uL proposed Lahouel *et al.*[39].

At the rats that were addicted to high doses of mercury and the combination of high doses of cadmium and mercury treated with vitamin C, erythrocyte regeneration was remarkable in batches (Dc and Fc). Similar results were observed by Lahouel *et al.*[39] in Wistar rats intoxicated with paracetamol and treated with propolis, and by Veena *et al.*[37] in mice intoxicated with lead nitrate and treated with ethanoic extract extra coriander.

Regarding hemoglobin and haematocrit, a similar trend was obtained with the lowest levels in the rats of group D and F. The values of these haematological parameters were increased due to treatment with vitamin C. Everything seems to confirm the conclusion Friot and Calvet[40] who argue that the factors that affect the general condition of the animal such as nutritional status, fluid balance and status influence pathological haematocrit.

Regarding the white blood cells, changes were observed mainly in rats lots D and F in which the number was lower. These low values were increased in rats lots Dc and Fc treated with vitamin C would be related to the toxic action of mercury and cadmium and mercury combination can induce leucopenia and thrombocytopenia in cases of severe liver dysfunction[41].

The significant decrease in hemoglobin (10.37±1.97 and 9.65±0.77 g/dl) in rats of group D and F, associated with a decrease in MCHC (27.35±1.39 and 27.00±0.70 g/l) indicate a tendency to macrocytosis and hypochromia hematopoiesis in the liver which occurs efficiently.

This decrease in blood cells has been corrected in rats and lots Dc Fc due to the favorable action of vitamin C used as a dietary supplement. Vitamin C has antioxidant potential which was confirmed by Szeto *et al.*[42] which states that the high amount of vitamin C in pineapple comus contributes to over 30% of its potential antioxidant, which promotes its favorable action on the liver in the regulation of hematopoiesis.

This decrease in hemoglobin was also found by Bersenyi *et al.*[43] in rabbits poisoned by lead, by Sinha *et al.*[44] in mice exposed to cadmium chloride, for Ognjanović *et al.*[45] in rats exposed to cadmium chloride. This result deferred of Smaoui *et al.*[46] which exposed rats to the exhaust gas which had induced erythropoiesis by hypoxia. Mercury and the combination of high concentrations of cadmium and mercury could inhibit heme synthesis of red blood cells and cause anemia signs described by Bottomley and Muller-Eberhard[47]. These signs are offset by the beneficial effect of vitamin C. However, red blood cells are low in heme, where the decline in MCHC, already indicated by our results in rats of Lot D and F. We also found that the rate Red blood cell, White blood cell, Hemoglobin, Haematocrit, the mean corpuscular hemoglobin concentration were not statistically different in control rats (G) and those addicted and treated (lots Dc and Fc). This could be explained by the antioxidant role of vitamin C. It has the ability to capture and deactivate free radicals. According to Siess *et al.*[48], it acts by preventing the binding of free radicals on DNA by activation of detoxification and protection of the capillary walls as noticed Kawabata *et al.*[49].

Awodele *et al.*[22], reported that the administration of vitamin C (8 mg/kg) corrected some of the potential harmful rifampicin of the deoxyribonucleic acid (DNA) in mice. This is due to the small dose lower than that used in this study (150 mg/kg). Ognjanović *et al.*[45] have also shown that as our of results pretreatment with vitamin E and C showed a protective role on the toxic effects of cadmium on haematological values, lipid peroxide. Similar results were obtained by Fox *et al.*[50], which showed the protective effect of vitamin C on anemia induced by heavy metals in rats.

Conclusion

These findings suggest that cadmium and mercury present in the environment and in particular in foodstuffs of first necessity cause of haematological disturbances in the blood. However, co-administration of (cadmium and mercury) and antioxidant (vitamin C) has protective effect hématotoxic due to cadmium and mercury. It could be concluded from the present study that vitamin C has potent antioxidant activity against cadmium and

mercury sensitive Haematological. It may then be recommended its use as a dietary supplement to rid the body of these xenobiotics that affect the health of the population. The consumption of rich foods in vitamin C is also highly recommended.

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