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Reversal of cadmium induced toxicity following dietary supplementation with garlic, ginger and cabbage in male Wistar rats

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ABSTRACT

The effect of dietary supplementation with garlic, ginger and cabbage at the rate of 10% of the feed on cadmium induced toxicity was investigated in male Wistar rats. Cadmium chloride in a single acute dose of 3 mg Cd/kg body weight was administered subcutaneously to six study groups (n = 10 per group), which were thereafter placed on test diets as follows: rat chow and water (group 1) which served as normal control and was neither exposed to cadmium nor antioxidants, group 2 was administered Cd and served as Cd treated control, group 3 received rat chow supplemented with vitamin E, group 4 received rat chow supplemented with garlic, group 5 was given rat chow supplemented with cabbage whereas group 6 had rat chow supplemented with ginger. At the end of 28 days feeding period, the animals were sacrificed and blood obtained for hormonal, biochemical and haematological analyses. It was observed that Cd administration induced 75-78% increase in total acid phosphatase (TAP) and prostatic acid phosphatase (PAP) activities respectively and 22% increase in alkaline phosphatase (ALP) activity relative to control. Serum TAP, PAP and ALP activities in all the supplemented groups were significantly reduced ($p < 0.05$) relative to the Cd treated, non-supplemented animals. Tendency towards anemia (decreased % PCV, [Hb] and RBC counts) in Cd treated group 2 was reversed as evidenced by significant increase ($p < 0.05$) in PCV, [Hb] and RBC counts in supplemented groups. Serum testosterone which was reduced as a result of Cd treatment was restored to normal levels as a consequence of supplementation.

Key words: Cadmium toxicity, Antioxidant supplements, Ginger, Cabbage, Garlic.

INTRODUCTION

Cd is an established toxic and carcinogenic heavy metal pollutant [1]. It is used in various chemical forms in the metallurgical and other industrial processes such as the production of pigments, batteries and reagents [2]. Environmental exposure to Cd can occur through the diet

and drinking water [3] or by Cd fume inhalation [1,4]. The element accumulates predominantly in the liver and kidney [5]. Hepatotoxicity is reported to be a major cause of acute Cd lethality [6]. The reaction mechanism is proposed to involve the generation of reactive oxygen species (ROS) [7]. Antioxidants such as vitamin C, E and Selenium have been demonstrated to counter free radical generation by Cd [8].

The present study explores the possibility of ameliorating the toxicity of Cd by using natural food spices and vegetable with proven antioxidant capacities such as ginger [9], garlic [10] and cabbage [11-12].

MATERIALS AND METHODS

Food spices and vegetable:

Two hundred grams (200g) each of garlic, cabbage and ginger were obtained from Marian Market in Calabar, Cross River State, Nigeria and conveyed to the Laboratory Facility of Biochemistry Department, University of Calabar, Calabar, Nigeria. They were washed, cut into small sizes, shade dried and made into fine powder using a manual mechanical grinder.

Experimental animals and treatment protocol:

Sixty (60) male albino Wistar rats weighing 140-220 g were obtained from the animal house facility of Biochemistry Department, University of Calabar, Calabar, Nigeria. The animals were acclimatized for two weeks on normal rat chow (Guinea Feeds Nigeria Limited, Benin City, Nigeria). Water was provided ad libitum. They were maintained under standard housing conditions of adequate ventilation with room temperature of 28±2^{o}C, relative humidity 46±5% and twelve hour light – dark regimen. Generally, the study was carried out in accordance with the guidelines of the Institutional Animal Ethics Committee. The experimental animals were divided into six study groups (10 animals per group) and treated as follows:

Group 1*: was administered normal diet (rat chow)*
Group 2*: was administered a single dose of 3mg Cd/kg body weight subcutaneously in addition to normal diet and served as Cd treated control.*
Group 3*: was administered a single dose of 3 mg Cd/kg body weight and vit. E.*
Group 4*: was administered a single dose of 3 mg Cd/kg body weight and diet containing 10 g garlic powder per 100 g of feed.*
Group 5*: was administered a single dose of 3 mg Cd/kg body weigh and diet containing 10 g cabbage powder per 100 g of feed.*
Group 6*: was administered a single dose of 3 mg Cd/kg body weight and diet containing 10 g ginger powder per 100 g of feed.*

All animals were allowed free access to drinking water. At the end of the experimental period (28 days), the animals were anaesthetised in chloroform vapour, dissected and blood samples collected by cardiac puncture into sample bottles. The blood was allowed to clot for three hours at about 4^{o}C. Serum was obtained by centrifugation at 10,000 RPM for five minutes using a bench top centrifuge.

Biochemical Analyses

Serum alkaline phosphatase (ALP) was assayed using the Reflotron assay kit while total acid phosphatase (TAP) and prostatic acid phosphatase PAP were determined using the Randox Kit. Haematological assessment of blood for haemoglobin concentration, packed cell volume (PCV), WBC and RBC counts were carried out as described by Lewis et al. [13]. Testosterone was assayed using the micro well ELISA Kit [14].

Statistical Analysis

Results were expressed as Mean ± SD. Statistical analyses was carried out using the one way analysis (ANOVA). The students t-test was used for pair wise comparison of means. Statistical significance was accepted at $p < 0.05$).

RESULTS

The effects of treatment on enzyme parameters, serum testosterone level and haematological indices are summarized in Tables 1 – 2. There were significant elevations in serum total acid phosphatase (TAP), prostatic acid phosphatase (PAP) and alkaline phosphastase (ALP) activities in the Cd treated group compared with the antioxidant supplemented groups (Table 1). Values for Cd treated rats showed about 75% increase in serum TAP, 78% increase in PAP and 22% increase in ALP activity compared with the values obtained in the control. In all the Cd treated and antioxidant supplemented groups, the values of TAP, PAP and ALP were reduced compared with Cd treated non –supplemented group. Cd administration also resulted in a significant decrease ($p < 0.05$) in the serum level of testosterone. This decrease was reversed in all the antioxidant supplemented groups (groups 3 to 6). Table 2 shows the effects of Cd administration and dietary supplementation with antioxidants on haematological indices. The packed cell volume (PCV), haemoglobin concentration [Hb], and RBC counts in the Cd treated rats were significantly reduced compared with the control group. In all the groups treated with antioxidants there was a significant increase ($p < 0.05$) in their respective PCV, [HB] and RBC count compared with the Cd treated group. The WBC Count increased non-significantly as a result of Cd administration and remained unaffected by antioxidant supplementation.

Table 1. Effect of cadmium administration and dietary supplementation with vitamin E, garlic, cabbage and ginger on total acid phosphatase (TAP), prostatic acid phosphatase (PAP), alkaline phosphatase (ALP) activities and testosterone levels (Mean±SD) in male Wistar rats

Treatment Groups (n=10/group)	Enzyme Activity (U/L)			Testosterone (ng/ml)
	TAP	PAP	ALP	
Group 1 (Control)	6.05 ± 0.26	5.10 ± 0.04	50.80 ± 0.57	1.23 ± 0.04
Group 2 (Cd treated)	10.60 ± 0.07	9.20 ± 0.06	62.00 ± 0.14	0.76 ± 0.06
Group 3 (Cd+Vit E)	4.80 ± 0.07[a]	4.30 ± 0.03[a]	53.20 ± 0.49[a]	1.40 ± 0.01[a]
Group 4 (Cd+Garlic)	5.45 ± 0.35[a]	4.75 ± 0.02[a]	51.40 ± 0.63[a]	1.60 ± 0.10[a]
Group 5 (Cd+Cabbage)	5.95 ± 0.20[a]	5.25 ± 0.03[a]	51.30 ± 0.14[a]	1.12 ± 0.02[a]
Group 6 (Cd+Ginger)	4.65 ± 0.07[a]	4.40 ± 0.01[a]	54.60 ± 0.50[a]	1.04 ± 0.06[a]

[a] indicates significant difference ($p < 0.05$) in result of Cd treated and antioxidant supplemented rats compared with group 2 (Cd treated without supplement).

Table 2. Effect of cadmium administration and dietary supplementation with vit. E, garlic, cabbage and ginger on haematological indices (Mean±SD) in male Wistar rats

Treatment groups (n=10/group)	[Hb] (g/l)	PCV (%)	WBC ($10^6/mm^3$)	RBC ($10^6/mm^3$)
Group 1 (Control)	10.68 ± 0.41	35.0 ± 0.22	5.80 ± 0.41	7.28 ± 0.21
Group 2 (Cd treated)	6.75 ± 0.38	22.0 ± 0.60	7.10 ± 0.52	2.97 ± 0.29
Group 3 (Cd+Vit E)	9.61 ± 0.32[a]	32.0 ± 0.38[a]	7.70 ± 0.26	6.48 ± 0.41[a]
Group 4 (Cd+Garlic)	9.28 ± 0.54[a]	30.0 ± 0.51[a]	6.90 ± 0.46	6.09 ± 0.61[a]
Group 5 (Cd+Cabbage)	9.50 ± 0.3[a]	31.0 ± 0.31[a]	7.30 ± 0.50	6.31 ± 0.12[a]
Group 6 (Cd+Ginger)	9.20 ± 0.41[a]	30.0 ± 0.40[a]	6.40 ± 0.40	6.01 ± 0.43[a]

[a] indicates significant difference ($p < 0.05$) in result of Cd treated and antioxidant supplemented rats compared with group 2 (Cd treated without supplement).

DISCUSSION

In recent times, attention has been focused on the physiological importance of a wide variety of naturally occurring polyphenol compounds that act as antioxidants [15]. These compounds are found abundantly in plants including ginger [9], garlic [10] and cabbage [11-12]. They exert profound chemo-preventive activities due to their ability to scavenge and reduce the production of free radicals and act as transition metal inhibitors [15-16]. Cd toxicity has been proposed to involve the generation of reactive oxygen species [7]. Antioxidant nutrients such and as vitamin C, E and Selenium have been found to counter free radical generation by Cd [8]. The present study was designed to evaluate the protective role of ginger, cabbage and garlic against Cd toxicity.

Cd administration caused an increase in total acid phosphatase, prostatic acid phosphatase and alkaline phosphatase activities (Table 1). Alkaline phosphatase activity is present in most organs of the body and is especially associated with membranes and cell surfaces located in the mucosa of the small intestine, proximal convoluted tubule of the kidneys, bone (osteoblasts), liver and placenta. An elevation in serum alkaline phosphatase activity commonly originates from the liver and bone. Its measurement is of particular interest in the investigation of hepatobiliary and bone disease associated with increased osteoblastic activity [17]. Induction of alkaline phosphates synthesis is the usual response of the liver to any form of biliary obstruction [18]. The increase in alkaline phosphatase, total and prostatic acid phosphatase activities represent general hepatic toxicity [17,19] and specific toxicity to the prostate gland [20-21] respectively. Waalkes [22] had earlier suspected an association between Cd exposure and prostate cancer. The generalised increase in phosphatase enzyme activities observed in this study could be attributed to Cd toxicity. The transgenerational toxicity effects of cadmium had been described [23]. In this study, dietary supplementation with vitamin E, ginger, garlic or cabbage triggered a reduction in enzyme activities towards the control values. Obianime and Roberts [8] had also observed that vitamin E reverses the Cd induced increase in phosphatase enzyme activity.

Testosterone is an important androgen secreted by the testes. It is essential for the development of male sexual characteristics and spermatogenesis. Exposure of male rats to cadmium caused a reduction in testosterone levels (Table 1). This could be attributed to testicular damage. Benoff et al. [24] demonstrated that cadmium and lead accumulate in the male reproductive organs causing alterations in hormonal concentrations as well as fertility and sperm parameters. Cd toxicity has also been implicated in the high rate of infertility among Nigerian men [25]. Dietary supplementation with garlic, ginger or cabbage resulted in a significant improvement in hormonal level probably as a consequence of their high content of antioxidants.

Anemia is an important manifestation of cadmium toxicity [1]. Cd induced anemia has been attributed to an impairment in the synthesis of erythropoietin, a hormone whose function is to promote formation of the red blood cells [26]. Various haematological parameters were evaluated in this study (Table 2). The PCV, [Hb] and RBC count in the Cd treated rats were observed to be significantly reduced ($p < 0.05$) compared to control. Wilson et al. [27] noted that rats develop anemia when exposed to dietary Cd levels as low as 31 ppm. Friberg et al. [28] had observed anemia in humans as a consequence of environmental exposure to Cd. The liver, spleen and bone marrow are the major haematopoietic organs which serve as targets of Cd exposure [29]. The present study has, however, demonstrated that Cd induced anemia can be reversed following dietary supplementation with antioxidant rich spices and vegetable.

The WBC count is regarded a non-specific predictor of various pathologic conditions including stress [13]. There was a non-significant increase in the WBC count for all the Cd treated and antioxidant supplemented rats. This observation, however, lacks clinical significance.

CONCLUSION

Exposure to Cd has been demonstrated to alter the activities of specific enzymes, haematological and hormonal indices in male rats. These account for the toxicity of this element to the reproductive and haematopoietic tissues. Dietary supplementation with ginger, garlic and cabbage which have antioxidant properties reversed the alterations in biochemical parameters and thus ameliorate the toxic effects of Cd.

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