

Full Length Research Paper

Ameliorative effect of cabbage extract on cadmium-induced changes on hematology and biochemical parameters of albino rats

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The effect of dietary supplement containing cabbage on cadmium – induced toxicity was studied in wistar rats. The effect of cadmium was investigated in 3 animals groups. Group A, rats were fed normal basal diet only. Group B rats were placed on normal basal, mixed with cadmium chloride (3 mg/kg body weight daily) while group C rats were fed with basal diet mixed with Cadmium chloride (3 mg/kg body weight) and 0.5 kg dry cabbage pellets daily. Rats were monitored for 28 days. At the end of the treatment, the animals were sacrificed using chloroform vapor. Oral rat LD50 for Cadmium Chloride, Anhydrous is 88 mg/kg. The effect of cadmium treatment alone and combined cadmium-cabbage treatment on lipid peroxidation, as measured by malondialdehyde levels in testes and kidney, serum activities of acid phosphatase (ACP) and prostatic acid phosphatase (PAP) and alkaline phosphatase (ALP) were investigated alongside testicular and kidney organ weight and assessment of some hematological indices. The result showed that cadmium induced a significant increase in both testicular and kidney malondialdehyde (MDA); but dietary cabbage seem to have a beneficial effect on lipid peroxidation. Cadmium also induced a 75% increase in ACP, 98% in PAP and 22% increase in ALP, but cabbage supplementation tended to produce a reduction in the activities of these enzymes ($p = 0.001$). Result of organ weight analysis in Cd –exposed rats showed a decrease in testes and kidney weight. Comparatively rats whose diet contained cadmium with cabbage supplementation showed an increase in organ weight. Administration of combined treatments of cadmium and cabbage may provide beneficial effects against cadmium-induced changes on the testicular and kidney weight, malondialdehyde, liver enzymes; alkaline phosphatase, acid phosphatase and prostatic acid phosphatase levels by reducing cadmium –associated oxidative stress.

Key words: Cabbage, cadmium, toxicity, amelioration.

INTRODUCTION

Cadmium is a heavy metal, which is widely used in industry, affecting human health through occupational and environmental exposure. In mammals, it exerts multiple toxic effects and has been classified as a human carcinogen by the International Agency for Research on Cancer. Cadmium affects cell proliferation, differentiation,

apoptosis and other cellular activities. Cadmium is particularly a concern in environmental pollution because it is said to accumulate in the human body causing renal dysfunction, pulmonary emphysema, kidney damage and osteoporosis (El-Demerdash et al., 2004).

Cadmium (Cd) is an environmental toxicant and an

endocrine disruptor in humans and rodents. Several organs are affected by Cd and recent studies have illustrated that the testis is exceedingly sensitive to Cd toxicity. More importantly, Cd and other toxicants, such as heavy metals ; lead, mercury, and estrogenic-based compounds like bisphenols may account for the recent declining fertility associated with reduced sperm count and testis function in men in developed countries (Siu et al., 2009). Cadmium induced toxicity has been shown to be alleviated by antioxidants; L -ascorbic acid (Shirashi et al., 1993), broccoli (Zoyne et al., 2008), natural anti-oxidant Garlic (Kumar et al., 2009) and naringenin (Renugadevi et al., 2009), which is naturally occurring citrus flavonone.

Previous study (Ola-Mudathir et al., 2008) demonstrated that aqueous extracts of onion and garlic could proffer a measure of protection against Cd-induced testicular oxidative damage and spermiotoxicity by possibly reducing lipid peroxidation and increasing the antioxidant defense mechanism in rats. The hepatoprotective effect of onion and garlic extracts on cadmium (Cd)-induced oxidative damage in rats has also been reported (Hilman, 2009). Anti cancer properties of cabbage have been documented *in vivo and vitro* (Michael et al., 1999). These including the inhibition of the formation of free radicals, support of endogenous radical scavenging mechanism, enhancement of cellular antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase), inhibition of low density lipoprotein from oxidation by free radicals and inhibition of the activation of the oxidant induced transcription factor and nuclear factor kappa (NP-KB) (Borek, 2001).

Cabbage is a member of the cruciferous family. Other vegetables that have developed or evolved from the early strains of cabbage include; brussels sprouts, cauliflower, kale and kohlrabi. Cabbage is a hardy vegetable that is available in various shades of green, as well as red or purple varieties. Most varieties have smooth leaves, but some types have ruffled textured leaves. The most popular varieties are green, red, savoy and Chinese. Cabbage is usually shredded into salads or used as an ingredient in stews, soups or baked dishes. Scientists at the American Cancer Society have found convincing evidence that diets high in cruciferous vegetables lower the risks of many forms of cancer (Byers et al., 2002). The present study was undertaken to assess if dietary

supplementation with cabbage has any ameliorating effect on acute cadmium toxicity in Wistar rats.

MATERIALS AND METHODS

Animals

Thirty albino rats of wistar strain weighing 140 – 220 g were purchased from the disease free stock of the Department of Biochemistry animal house, University of Port Harcourt and transferred to the Department of Biochemistry University of Calabar, where the study took place. They were acclimatized for two weeks on a commercial rat diet prior to experimentation. Permission and approval for the animal studies were obtained from the College of Medical Science Animal Ethics Committee of the University of Calabar. The rats were weighed and randomly assigned on the basis of their weights into three study groups (A, B and C) of ten rats each and were housed in plastic cages with wire screen tops. They were kept under adequate ventilation with room temperature and relative humidity of $29 \pm 2^\circ\text{C}$ and 40 - 70%, respectively, with a 12 h natural light-dark cycle. Food and water was provided *ad libitum*, and good hygiene was maintained by constant cleaning and removal of faeces with spilled feed from cages daily.

Animals in group A were fed normal basal diet only. Group B rats were placed on normal basal diet mixed with cadmium chloride (3 mg/kg body weight) daily while group C rats were fed with basal diet mixed with Cadmium chloride (3 mg/kg body weight) and 0.5 kg dry cabbage pellets daily. Experimental rats were monitored for 28 days. At the end of the treatment period, the animals were sacrificed using chloroform vapor. Oral rat LD50 for Cadmium Chloride, Anhydrous is 88 mg/kg. Blood was collected by cardiac puncture from all experimental rats into two tubes (a gel tube without anticoagulant and a tube containing Ethylene Diamine Tetra Acid (EDTA).

Sample in the gel tube was allowed to clot, centrifuged to obtain serum sample used for the colorimetric and enzyme immunoassay analysis of malondialdehyde level (MDA), alkaline phosphatase (ALP), acid phosphatase (ACP), and Prostatic acid phosphatase (PAP) using Randox kits (Randox Clinical Diagnostic solutions, UK). The EDTA anticoagulated sample was used for the analysis of hematological indices of hemoglobin (Hb), packed cell volume (PCV), white blood cells count (WBC) and red cell counts (RBC). Kidney and testes were surgically removed, weighed and placed in an iceberg and later trimmed to 5 g weight and further used to ascertain the extent of lipid peroxidation.

Statistics

Statistical analysis was carried out using a Statistical Package for Social Sciences version 10 (SPSS Inc., Chicago, IL.). Data collected were expressed as mean \pm SD and the student's t – test was used for analysis. Descriptive analysis of percentages of continuous variables was reported. Comparisons were assessed using mean and chi-square test. A p-value of < 0.05 was considered statistically significant in all statistical comparison.

RESULTS

Malondialdehyde level (MDA) was used as an index of lipid peroxidation in testes and kidney homogenates of controls, and experimental animals treated with cadmium and cabbage supplements as shown in Table 1. The mean \pm SD values of MDA levels obtained for the testes

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Abbreviation: MDA, Malondialdehyde; ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase; ACP, acid phosphatase prostatic; PAP, acid phosphatase; EDTA, ethylene diamine tetra acid; NAC, n-acetyl cysteine; MiADMS, monoisoamyl 2, 3-dimercaptosuccinate; Hb, blood hemoglobin; TEC, total erythrocytic count; PCV, packed cell volume; TLC, total leukocyte count.

Table 1. Malondialdehyde levels in testes and kidney of controls and cadmium treated rats.

Treatment group	Testes $\mu\text{mol/L}$	Kidney $\mu\text{mol/L}$	P-value
Group A (control)	2.14 \pm 0.03	2.10 \pm 0.07	0.01
Group B (Cd-treated)	3.42 \pm 0.02	2.76 \pm 0.03	
Group C (Cd+cabbage)	1.45 \pm 0.07	2.53 \pm 0.03	

Values are mean \pm SD and indicates a statistically significant difference in the malondialdehyde levels in the testes and kidney of Cd treated rats compared to rats treated with Cd and cabbage supplementation.

Table 2. Total ACP, PAP, and ALP activities in control and cadmium exposed Wistar rats.

Treatment group	ACP (U/l)	PAP (U/l)	ALP(U/l)	P-value
Group A (Control)	6.05 \pm 0.26	5.10 \pm 0.04	59.80 \pm 0.57	0.001
Group B (Cd-treated)	10.60 \pm 0.07	9.10 \pm 0.06	62.00 \pm 0.14	
Group C(Cd+cabbage)	5.95 \pm 0.21	5.25 \pm 0.03	51.30 \pm 0.14	

Values are mean \pm SD and indicates a statistically significant difference between enzyme activities in Cd treated rats compared to rats treated with Cd and cabbage supplementation.

Table 3. Effect of Cd exposure and cabbage supplementation on testes weights of Wistar rats.

Treatment group	Testes weight (g)	P-value
Group A (Control)	2.12 \pm 0.14	0.001
Group B (Cd-treated)	1.90 \pm 0.16	
Group C (Cd+ cabbage)	2.87 \pm 0.17	

Values are mean \pm SD and indicates a statistically significant difference in the testes weight of Cd treated rats compared to rats treated with Cd and cabbage supplementation.

in various groups were 2.14 \pm 0.03 for controls, 3.42 \pm 0.02 for Cd-treated and 1.45 \pm 0.07 for rats fed with Cd supplemented with cabbage. Also, in the kidney tissue homogenates, the MDA levels of control and experiment groups were 2.10 \pm 0.07 for controls, 2.76 \pm 0.03 for Cd-treated and 2.53 \pm 0.06 nmol/L for rats fed with Cd supplemented with cabbage. Result shows that cadmium treatment increased the MDA level in the testes by 60% relative to the control that received placebo.

The result further indicated a significant decrease ($p = 0.01$) in MDA levels in cd-treated with cabbage supplemented groups. The result equally showed a 31% increase in kidney MDA levels for the groups that had cd-treatment alone. Comparatively, cabbage supplementation tended to have a beneficial effect associated with a decline in the level of kidney MDA. The mean \pm SD values of ACP for the control, Cd-treated and Cd-treated with cabbage supplement were 6.05 \pm 0.26, 10.60 \pm 0.07 and 5.95 \pm 0.21 U/l, respectively. The mean \pm SD values for PAP activities for the control, Cd-treated and Cd-treated with cabbage supplement were 5.10 \pm 0.04, 9.10 \pm 0.06 and 5.25 \pm 0.03 U/l, respectively. The values of ALP for control, Cd-treated and Cd-treated with cabbage

were 59.80 \pm 0.57, 62.00 \pm 0.14 and 51.30 \pm 0.14, respectively. The result showed that cadmium exposure significantly increased ACP, PAP and ALP activities in the cadmium fed rats, while cabbage supplementation tended to have a beneficial effect associated with a decline in the level of these enzymes as shown in Table 2.

There was a statistically significant difference in the values of testes weight of Control group A, Cd-treated group B and Cd-treated with cabbage supplements (group C), (2.12 \pm 0.14, 1.90 \pm 0.16 and 2.87 \pm 0.17 g respectively) $p = 0.001$ as shown in Table 3. The values for kidney weight although, marginally higher in the cabbage supplemented groups (1.33 \pm 0.15) compared to the Cd-treated rats (1.26 \pm 0.18 g) and control rats (1.75 \pm 0.31). The difference was however, not statistically significant ($p = 0.06$) as shown in Table 4.

The haematological parameters assessed were HB, PCV and total WBC counts. The mean \pm SD values are outlined in Table 5. The values for HB, PVC and WBC count for Cd-treatment group alone showed a significant reduction in comparison to the cabbage supplemented group. The value for total WBC count for Cd-group showed a significant increase compared to the cabbage supplemented group as shown in Table 5.

DISCUSSION

Cadmium is a well-known human carcinogen and a potent nephrotoxin. This study showed that Cd treatment induced as much as 60% of lipid peroxidation in the testes of test rats compared to control. This agrees with the report of Grupta et al. (1997) which indicated that Cd is an inducer of cell oxidative stress. Pre-treatment with cabbage reduced testicular MDA, significantly in rats fed with Cd-supplemented with cabbage compared to the

Table 4. Effect of Cd exposure and cabbage supplementation on kidney weights of Wistar rats.

Treatment group	Kidney weight (g)	P-value
Group A (Control)	1.75 ± 0.31	0.06
Group B (Cd-treated)	1.26 ± 0.18	
Group C (Cd+ cabbage)	1.33 ± 0.51	

Values are mean ± SD and indicates a non- statistically significant difference in the testes weight of Cd treated rats compared to rats treated with Cd and cabbage supplementation.

Table 5. Effect of Cd-treatment and cabbage supplementation on hematological parameters of albino rats.

Treatment group	Haemoglobin value (g/dl)	Paced cell volume (%)	White cell count (x10 ⁹ /L)	P-value
Group A (Control)	10.68 ± 0.41	35 ± 0.22	5.8 ± 0.41	0.001
Group B (Cd-treated)	7.28 ± 0.21	22 ± 0.60	2.97 ± 0.29	
Group C (Cd+ cabbage)	9.50 ± 0.30	31 ± 0.31	7.3 ± 0.50	

Values are mean ± SD and indicates a statistically significant difference in the hematological values of Cd treated rats compared to rats treated with Cd and cabbage supplementation.

Cd-treated ones. Similarly, Cd-treatment alone enhanced lipid peroxidation in kidney tissues of Cd fed rats by as much as 31% compared to control. Pre-treatment with cabbage alongside with Cd-treatment reduced significantly the MDA levels compared to the Cd-treated ones. These findings are consistent with the report of Fleischer et al., 2001, which reported that cabbage may have some anti cancer activity. Also anti cancer and antioxidants properties of the cabbage –family vegetable have been documented *in vivo and in vitro* (Michael et al., 1999).

The effect of cabbage on serum ACP, PAP and ALP activities of rats fed with a basal diet mixed with cadmium chloride (3 mg Cd/kg) revealed that Cd -treatment can cause a significant increase in serum ACP, PAP and ALP activity in comparison to control values. Cd-treated rats showed 75% increased in ACP, 78% in PAP and 22% in ALP compared to the values for untreated controls. Cadmium accumulation in blood affects the renal cortex and causes renal failure. The increased activity of these enzymes may be as a result of leakage of these enzymes into blood stream due to the compromised integrity of the testes and kidney resulting from cadmium toxicity. This finding is consistent with the report of Fouad et al. (2009) which indicated that Cd-induced oxidative damage in rat liver is amenable to attenuation by high dose of onion and moderate dose of garlic extracts possibly via reduced lipid peroxidation and enhanced antioxidant defense system that is insufficient to prevent Cd-induced hepatotoxicity. Cd was found to cause a marked increase in the levels of lipid peroxidation and glutathione S-transferase in Cd fed rats. They also observed a decrease in hepatic activities of alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase and a concomitant increase in the plasma activities of ALT and AST. We observed a reversal in the cadmium-induced increase in the activities of these

enzymes in the cabbage supplemented group. Supplementation with cabbage seems to play a protective role against cadmium-induced increases in ACP, PAP and ALP activities in rats. Cabbage seems to have antioxidant effect against cadmium induced toxicity.

Previous reports indicates that cadmium induced toxicity has been alleviated by antioxidants; L -ascorbic acid (Shirashi et al., 1993), broccoli (Zoyne et al., 2008) natural anti-oxidant Garlic (Zoyne et al., 2008; Kumar et al., 2009), naringenin (Renugadevi et al., 2009), a naturally occurring citrus flavonone. Similarly, aqueous extracts of onion and garlic has been shown to proffer a measure of protection against Cd-induced testicular oxidative damage and spermiotoxicity by possibly reducing lipid peroxidation and increasing the antioxidant defense mechanism in rats (Ola-Mudathir et al., 2008). Antioxidant properties of cabbage seem to have the ability to mop up free radicals. Cabbage is rich in antioxidant nutrients, which play an important role in health maintenance. They neutralize harmful chemicals called "free-radicals" that cause cell damage in the body. Antioxidants have been strongly linked to the protection from numerous diseases; heart disease, cancer, eye disease as well as the regulation of the immune system. In addition, cabbage contains beta-carotene, lutein and zeaxanthin, carotenoids that are a large class of natural plant pigments. They have chemo-protective effects, exhibit strong antioxidant properties and may reduce the risk of age-related macular degeneration and some types of cancer (Michael et al., 1999; Lynn et al., 2006).

Our study indicated that testes weight was significantly lower in Cd- treated rats in comparison to rats feed with Cadmium supplemented with cabbage extract. This finding is in agreement with previous reports (Ola-Mudathir et al., 2008; Fouad et al., 2009) which indicated that Cd caused a marked rise in testicular lipid

peroxidation (LPO) and glutathione S-transferase (GST) levels. Cd intoxication significantly decreased epididymal sperm concentration and sperm progress, motility, increased percent total sperm abnormalities and live/dead count. They also demonstrated that aqueous extracts of onion and garlic could proffer a measure of protection against Cd-induced testicular oxidative damage and spermotoxicity by possibly reducing lipid peroxidation and increasing the antioxidant defence mechanism in rats. The mechanism of cadmium-induced testicular toxicity is poorly understood. Previous studies focusing on cadmium-related changes in testicular histopathology have implicated testicular blood vessel damage as the main cause of cadmium toxicity (Jian-Ming et al., 2003).

Although, not statistically significant, our study indicated that kidney weight was significantly lower in Cd-treated rats in comparison to rats feed with cadmium supplemented with cabbage extract. We observed that, cabbage supplementation produced a beneficial effect on the kidney weight. The degree of cadmium toxicity might depend on the extent of free radical load within the testes and kidney. Previous study by Sinha et al., 2009 investigated the protective role of taurine against cadmium-induced oxidative impairment in murine liver and observed that cadmium-induced hepatic oxidative stress and necrosis was prevented by the prophylactic properties of taurine. Similarly, the influence of an antioxidant agent such as N-acetyl cysteine (NAC) or mannitol on the cadmium chelating ability of monoisoamyl 2, 3-dimercaptosuccinate (MiADMS) was investigated in cadmium pre-exposed rats by Tandon et al., 2003. The combined treatments also improved liver and brain endogenous zinc levels, which were decreased due to cadmium toxicity.

The results suggest that, the administration of an antioxidant during chelation of cadmium may provide beneficial effects by reducing oxidative stress. Similarly, the administration of thiamine during chelation therapy in cadmium poisoning has been shown to have a beneficial effect and more effective than thiol chelating agents alone (Tandon et al., 2000). Also, the influence of vitamin E supplementation on the burden of cadmium was investigated in Cd-exposed rats in a previous report (Tandon et al., 1999). The treatment with MFA-vitamin E or CaNa₃ DTPA-vitamin E was more effective than either vitamin E or chelating agent alone, in depleting blood and tissue Cd. The combined treatment showed an advantage over the individual agent in restoring Cd-induced biochemical changes. The treatment with chelator-vitamin E concomitantly with the exposure to Cd was more effective than post-Cd exposure treatment. Other antioxidants; S-adenosylmethionine, lipoic acid, glutathione, selenium, zinc, N-acetylcysteine (NAC), methionine, cysteine, alpha-tocopherol, and ascorbic acid have also been shown to have specific roles in the mitigation of cadmium toxicity (Patrick, 2003).

The effect of Cd-treatment and Cd-treatment with

cabbage on haematological indices and pathophysiological fitness of albino rats revealed a significant distortion of the haemopoetic parameters by Cd and a distinct alleviation of cadmium-associated distortion in the hematological indices by cabbage supplementation. The red blood cells showed marked hypochromasia, with burr, target and crenated red blood cells. The differential leukocyte count showed marked neutropenia and basophilia. This finding is consistent with previous study (El-Demerdash et al., 2004) which indicated that treatment with cadmium chloride caused a significant decrease in blood hemoglobin (Hb), total erythrocytic count (TEC) and packed cell volume (PCV), while total leukocyte count (TLC) increased. This report suggest that, the administration of combined treatments of cadmium and cabbage may provide beneficial effects against cadmium-induced changes in the testicular and kidney weight, malondialdehyde, liver enzymes; alkaline phosphatase (ALP), acid phosphatase (ACP), and prostatic acid phosphatase (PAP) levels by reducing cadmium-associated oxidative stress.

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