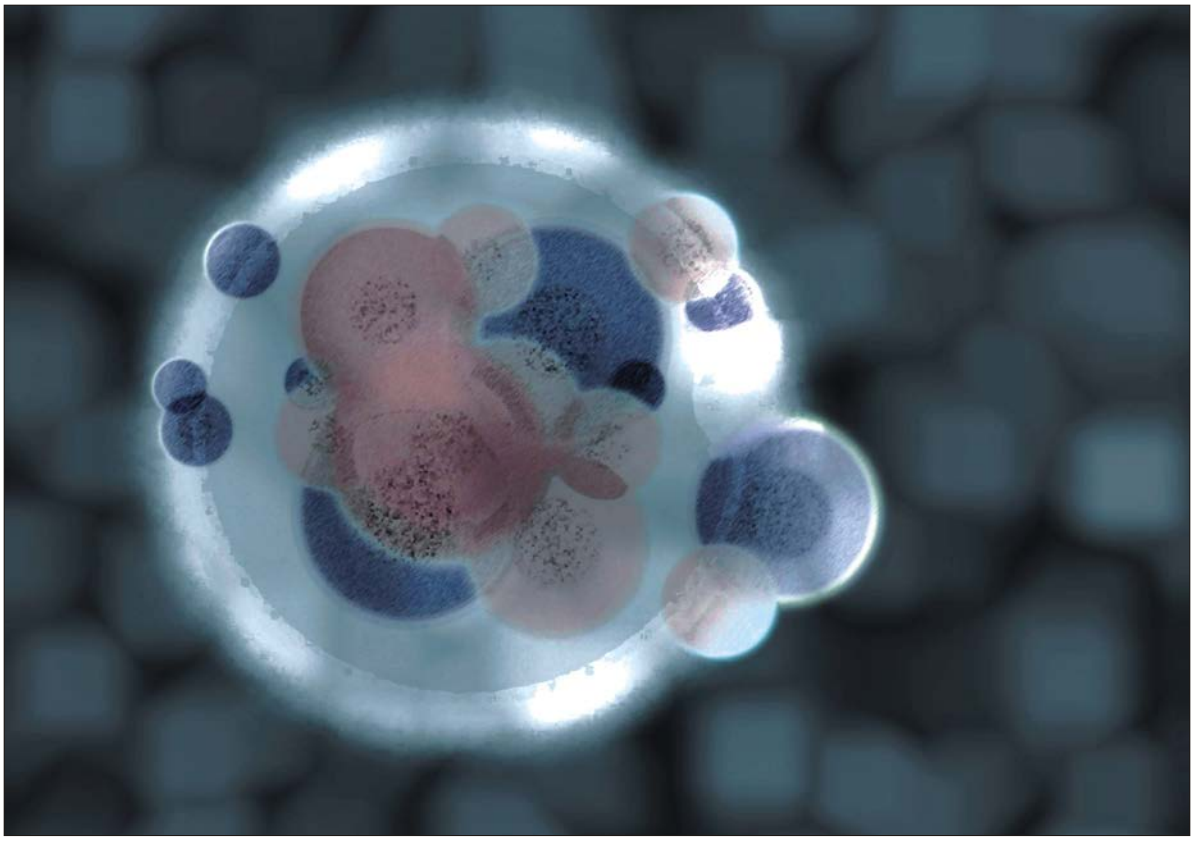

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Changes in Brain, Liver and Kidney Lipid Peroxidation, Antioxidant Enzymes and ATPases of Rabbits Exposed to Cadmium Ocularly

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Abstract: This study examined the effect of supplementing rabbit diet with Palm Oil (PO) on lipid peroxidation, antioxidant enzymes and ATPases of the brain, liver and kidney in ocular cadmium toxicity. New Zealand male rabbits were randomly assigned to 4 groups of 5 rabbits in a study that lasted for 4 weeks. A control group was given deionised water as eye drops and the other groups of rabbits were given eye drops of solution of 2 mg kg^{-1} body wt. cadmium (as $3CdSO_4.8H_2O$). The control and a test group were fed with the normal chow and animals in the other test groups were fed with the chow fortified with either 5% or 10% palm oil. Treatment of the rabbit with cadmium significantly ($p<0.05$) reduced their weight compared with the control and PO supplementations of the diets improved the weights of the animals. Brain, liver and kidney levels of lipid peroxidation were raised by cadmium and PO significantly ($p<0.05$) reduced the level of lipid peroxidation of these tissues. Cadmium significantly ($p<0.05$) reduced antioxidant enzymes and ATPases in the tissues compared with the control. Feeding the rabbits with PO significantly ($p<0.05$) increased the activities of these enzymes to levels comparable with the control, with the 10% supplementation producing a more pronounced effect. The study shows that PO can alter brain, liver and kidney antioxidant enzymes and ATPases in ways which suggest that it offers protection of the rabbits from ocular exposure to cadmium.

Key words: Cadmium, brain, liver, kidney, lipid peroxidation, ATPase, superoxide dismutase, catalase

INTRODUCTION

Cadmium posses a threat to humans because it can bioaccumulate in tissues and cause damage (WHO, 1992). Though the primary route of exposure for the general population is through the diet, it can be contacted through cigarette smoke. Avunduk *et al.* (1997) reported that cadmium accumulates in the lenses of rats exposed to tobacco smoke and that it causes cataracts. In humans, smoking over a pack of cigarettes a day increases the risk of developing cataract (Christen and Seddon, 1993; Hammond *et al.*, 1999).

Cadmium toxicity arises from the generation of free-radicals (Bagchi *et al.*, 1996) which cause oxidative stress and lipid peroxidation leading to damage of tissues (Kowalczyk *et al.*, 2003; Khan *et al.*, 2001; Antunes *et al.*, 2001; Freeman and Crapo, 1982). Cadmium also inhibits antioxidant enzymes which protects tissues by either binding to sulfhydryl groups essential for the enzymes, replace the bivalent metals like zinc, copper and manganese required by the enzymes, or decreases the bioavailability of selenium required by the enzymes (Timbrell, 1991). Another key sulfhydryl group attacked by cadmium is found in the enzyme Na^+/K^+-ATPase. This enzyme is involved in maintaining resting potential and the viability of cells. Carotenoids are some of the important dietary antioxidants (Burton and Ingold, 1984; Kennedy and Liebler, 1992) scavenging peroxyl radicals and preventing auto-oxidation (Bendich, 1993). Studies have shown a significant association between high intake of carotenoid-rich diets and lowered risk of cataracts (Knekt *et al.*, 1992). There is evidence that β-carotene lowers mortality risk of smokers (Greenberg, 1993). Palm oil is red and is rich in β-carotene and it is commonly consumed in Southern Nigeria. One hundred grams of the oil is known to contain 27, 280 μg β-carotene equivalent (Gaydou *et al.*, 1982) and the oil is readily digestible and absorbed (Eriyamremu and George, 1997). This oil constitutes the most source of β-carotene in the population of these Nigerians. The metabolism of oils and the handling of cadmium mostly involve the liver and kidney. As smokers can contact cadmium through the eyes, the heavy metal could also affect other organs. There are few studies on the role of ocularly exposed

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cadmium on autooxidation and anti-oxidant enzymes of organs and fewer still have examined the effect of palm oil as a natural source of β-carotene on liver, brain and kidney in ocular cadmium toxicity. The aim of this study is to determine the role of palm oil (rich in β-carotene) on autooxidation and anti-oxidant enzymes of the brain, liver and kidney in ocular cadmium toxicity.

MATERIALS AND METHODS

The animals used in this study were male New Zealand rabbits and weighed between 800 and 1200 g. They were purchased from the University of Benin animal farm, Benin, Nigeria. The animals were maintained on normal chow (product of Pfizer Feeds Nigeria Ltd.) and water *ad libitum* throughout the period of study. After a period of 2 weeks acclimatization to our laboratory conditions they were weighed and randomly assigned to 4 groups of 5 rabbits each such that the weight difference between the groups was about 5.0 g. They were thereafter weighed once a week throughout the study period and the feed intake and faecal output were measured daily. The first group of animals which served as the control were given deionised water as eye drops and fed on the normal chow. The other three groups of rabbits were given eye drops of solution of 2 mg kg^{-1} body wt cadmium (as $CdSO_4.8H_2O$, a product of Sigma, USA) and these animals acted as the test animals. The solution of the heavy metal was formed by dissolving $CdSO_4.8H_2O$, in distilled deionised water. One group of the test rabbits were fed with the normal chow alone and the other two test groups of rabbits were fed with the chow fortified with either 5 or 10% PO. The palm oil was from the Nigerian Institute for Oil Palm Research (NIFOR), Nigeria. Four weeks after the first cadmium administration the animals were sacrificed after been anaesthetized with chloroform and the brain, liver and kidney were excised. In all the treatments the animals were handled in accordance with the principles of laboratory animal care as contained in NIH guide for laboratory animal welfare-Vol. 3, 1985. The tissues were rinsed with normal saline and the biochemical assays were immediately carried out. Where the tissue could not be immediately assayed; they were placed in labeled containers and stored frozen at -20°C until required for biochemical assays which was within 48 h.

Digestion of samples: Before use, all glass and plastic utensils were washed in dilute nitric acid and then rinsed with deionised water. Weighed samples of the brain, liver and kidney of each group were digested separately in beakers with 20 mL of acid mixture ($HNO_3/HClO_4$; 4:1 v/v). The digestion was facilitated by heating at 100°C after which the sample was allowed to cool and thereafter diluted with deionised water to give a final volume of 100 mL.

Cadmium analysis: The cadmium concentrations in the digests were measured by atomic absorption spectrophotometry (Varian AA 1475 Spectrophotometer). The test metal was dissolved in deionised water and used as standard. The analytical methodologies on the samples were calibrated with the analysis of International Atomic Energy Agency (IAEA) reference biological sample V-10 (Hays). The cadmium concentration obtained for the reference sample was in agreement with the certified value. In all the determinations, blanks were prepared to determine the effect of reagent purity on the metal levels.

Tissue preparation: For the analyses of Superoxide Dismutase (SOD) and catalase activities, weighed portions of the tissues were homogenized in phosphate buffer (pH 7.0) and for ATPases activities, weighed samples were homogenized with physiological saline. The respective homogenates were centrifuged at 4000 g for 10 min under cold conditions, while the residue was recovered in case of ATPase assay, the supernatant was recovered in the case for the analysis of SOD, catalase and lipid peroxidation.

Biochemical assays: Na^+/K^+, Mg^{2+} and Ca^{2+} ATPase were estimated by the method of Bonting (1970) as modified by Takeo *et al*. (1980) and Adam-Vizi and Seregi (1982) and the inorganic phosphate released in the assays were determined (Fiske and Subarrow, 1925). Superoxide Dismutase (SOD) activity of the samples was assayed according to the method described by Misra and Fridovich (1972). One unit of the enzyme was defined as the amount of the enzyme required for 50% inhibition of oxidation of epinephrine to adrenochrome in one minute. Catalase (CAT) activity was assayed by the method of Kaplan and Groves (1992) and was determined as residual H_2O_2 after incubation with the enzyme. Estimation of membrane lipid peroxidation was by the method of Gutteridge and Wilkins (1982). Values for Thiobarbituric Acid Reactive Substances (TBARS) are Reported as Malondialdehyde (MDA) and quantified using a Molar extinction coefficient of 1.5×10^5 mol cm^{-1} and expressed as mmole MDA g^{-1} tissue weight. Total protein of the tissues was determined by the method of Lowry *et al*. (1951).

Statistical analysis: The values are reported as means±SEM Statistical difference was determined using

ANOVA and differences in the means were tested by Duncan's multiple range tests (Sokal and Rohlf, 1969).

RESULTS

The exposure of rabbits to cadmium significantly ($p<0.05$) reduced the weights of the animals compared with the control. The supplementation of the diet of the animals exposed to cadmium with 10% PO caused only about 9% reduction in weight compared with the about 29% reduction recorded in the test control rabbits whose diets were not supplemented with PO. Evaluation of the data reveal that addition of PO to the diet also significantly ($p<0.05$) reduced the food intake and dry faecal output of the rabbits compared with the test control. This study shows that cadmium administered ocularly reduces the weight of rabbits and PO mitigates the loss of weight (Table 1).

Cadmium accumulated in the brain, liver and kidney of the rabbits exposed to cadmium compared to the control where cadmium was not detected in the tissues. We observed that in the animals fed PO, there was a decrease in the level of cadmium in the various tissues compared with the level observed in the animals fed the normal chow alone (Table 2). The quantity of PO fed to the animals also affected the amount of cadmium in the tissues. In the brain, there was about 63% decrease in the level of cadmium in the 10% PO fed animals compared with about 48% observed in the 5% PO fed rabbits. This effect of PO on tissue cadmium decreased from brain to liver and then kidney. While the higher level of PO in the diet resulted in only 27% reduction in kidney cadmium, the 5% PO resulted in 25% reduction. Lipid peroxidation, an indicator of toxicity, as measured by the level of malondialdehyde also followed the same trend. When the sham and the test control were compared, MDA was significantly elevated in the test control. The levels of lipid peroxidation, returned towards control values in the tissues of rabbits whose diets were supplemented with palm oil, with the 10% supplementation resulting in most reduction.

Cadmium administration significantly ($p<0.05$) reduced SOD and CAT activities in the brain, liver and kidney compared with the control given deionised water (Table 3). This observed reduction in these antioxidant enzymes failed to reach significant ($p>0.05$) levels for CAT activity in the kidney. The inclusion of increasing doses of PO in the animal diet resulted in a progressive increase in the activities of these enzymes from the depressed state towards normal. In the brain, the 47% decrease in the activity of SOD observed in the test control compared with the sham control, was successively reduced to 25 and 18% following the feeding of 5 and 10%

Table 1: Effect of palm oil on the food intake and weight gain of rabbits exposed to cadmium ocularly

	Experimental group			
Parameters	Control diet	Control diet+cadmium	Control diet+cadmium + 5% palm oil	Control diet+cadmium + 10% palm oil
Initial weight (g)	904.0±4.3	908.0±5.2	907.3±5.0	907.0±5.3
Final weight (g)	1385.0±10.6	1237.0±11.6	1286.0±12.4	1335.0±14.0
Weight gain (g day^{-1} rabbit^{-1})	3.4±0.3^{a}	2.4±0.4^{b}	2.7±0.3^{bc}	3.1±0.3^{ac}
Food intake (g day^{-1} rabbit^{-1})	59.0±4.5^{a}	52.7±5.7^{ab}	47.9±5.6^{bc}	42.4±5.0^{c}
Faecal output (g day^{-1} rabbit^{-1})	8.2±2.3^{a}	7.9±2.4^{a}	6.1±1.6^{a}	6.4±2.0^{a}

Values are Means±SEM, n = 5. Means of the same row followed by different letter(s) differ significantly ($p<0.05$)

Table 2: Effect of palm oil on cadmium and lipid peroxidation levels in sections of rabbit eyes exposed to cadmium ocularly

	Experimental group			
Parameters	Control diet	Control diet+cadmium	Control diet+cadmium + 5% palm oil	Control diet+cadmium + 10% palm oil
Cadmium Level				
Brain	ND	3.3±0.3	1.7±0.4	1.2±0.4
Liver	ND	15.7±1.4	10.9±1.0	8.6±1.0
Kidney	ND	19.0±1.5	14.2±1.2	13.8±1.0
Malondialdehyde				
Brain	2.7±0.5^{a}	14.8±2.5^{b}	9.8±1.9^{c}	6.6±1.1^{d}
Liver	5.0±0.8^{a}	26.7±3.7^{b}	17.2±2.6^{c}	11.4±2.2^{d}
Kidney	3.0±0.6^{a}	36.0±4.1^{b}	25.1±3.6^{c}	20.0±3.5^{c}

Values are Means±SEM, n = 5. Means of the same row followed by different letters differ significantly ($p<0.05$); Values of cadmium in the tissues are expressed in μg Cd g^{-1} tissue. Lipid peroxidation is presented as mmole MDA g^{-1} tissue weight

Table 3: Effect of palm oil on brain, liver and kidney lipid peroxidation and antioxidant enzymes activities of rabbits exposed to cadmium ocularly

Parameters	Experimental groups: Control diet	Control diet+cadmium	Control diet+cadmium + 5% palm oil	Control diet+cadmium + 10% palm oil
Brain				
SOD	16.5±2.3^{a}	8.7±1.6^{b}	12.3±2.1^{abc}	13.4±2.0^{ac}
Catalase	7.8±1.2^{a}	5.2±1.3^{b}	5.0±1.0^{b}	4.4±1.2^{b}
Liver				
SOD	21.5±2.0^{a}	13.6±2.1^{b}	16.3±2.1^{c}	17.4±2.3^{ca}
Catalase	12.7±1.2^{a}	6.3±0.9^{b}	8.9±1.0^{c}	11.4±1.2^{a}
Kidney				
SOD	13.5±2.0^{a}	7.7±1.4^{b}	9.3±1.5^{b}	10.4±2.0^{ab}
Catalase	4.8±0.4^{a}	5.5±0.6^{a}	4.9±0.5^{a}	5.4±0.6^{a}

Values are Means±SEM, n = 5. Means of the same row followed by different letter(s) differ significantly ($p<0.05$); SOD activity is expressed as Units/g tissue; Catalase activity is expressed as μmole min^{-1} mg^{-1} tissue

Table 4: Effect of palm oil on brain, liver and kidney ATPases activities of rabbits exposed to cadmium ocularly

Parameters	Experimental groups: Control diet	Control diet+cadmium	Control diet+cadmium + 5% palm oil	Control diet+cadmium + 10% palm oil
Brain				
Na^{+}/K^{+} ATPase	85.3±5.2^{a}	62.9±4.2^{b}	75.4±4.8^{c}	80.7±5.1^{ac}
Mg^{2+} ATPase	76.5±4.8^{a}	64.7±5.1^{b}	72.3±6.1^{ab}	79.4±6.5^{ab}
Ca^{2+} ATPase	67.8±3.5^{a}	75.7±4.4^{b}	85.9±4.7^{c}	82.4±4.2^{bc}
Liver				
Na^{+}/K^{+} ATPase	94.2±4.9^{a}	70.1±5.2^{b}	63.4±4.2^{b}	70.9±5.1^{b}
Mg^{2+} ATPase	82.5±5.0^{a}	52.6±5.1^{b}	42.3±4.1^{c}	67.4±5.5^{d}
Ca^{2+} ATPase	71.2±4.5^{a}	61.3±4.4^{bc}	55.9±4.7^{b}	68.4±4.2^{ca}
Kidney				
Na^{+}/K^{+} ATPase	68.3±3.2^{a}	32.9±3.2^{b}	45.4±3.3^{c}	50.3±4.1^{c}
Mg^{2+} ATPase	56.5±3.0^{a}	28.7±3.1^{b}	42.3±3.1^{c}	47.4±4.0^{d}
Ca^{2+} ATPase	47.8±3.4^{a}	20.7±4.0^{b}	30.9±3.1^{c}	32.4±4.0^{c}

Values are Means±SEM, n = 5. Means of the same row followed by different letter(s) differ significantly ($p<0.05$); ATPase activity is represented as μmol Pi released min^{-1} mg^{-1} protein

PO, respectively. The about 50% reduction of the activity of liver CAT observed in the test control compared with the sham control, changed to only a 10% reduction in activity when 10% PO was fed to the rabbits. This study thus shows that the antioxidant enzymes SOD and CAT of the brain, liver and kidney are responsive to palm oil in the diet.

Compared with the control animals treated with deionised water, cadmium treatment of rabbits significantly ($p<0.05$) reduced the activities of Na^{+}/K^{+}, $Mg^{2+}$and Ca^{2+}-ATPases of the brain, liver and kidney. Feeding the rabbits with diets either supplemented with 5 or 10% palm oil raised the activity of the enzymes in these tissues compared with the test control with the 10% supplementation producing a more pronounced effect than the 5% one. Again this report shows that palm oil in the diet of rabbits alter ATPases of the some tissues (Table 4).

DISCUSSION

Cadmium has been shown to be contacted through different routes and it is present in cigarette. Cigarette smoke is not only inhaled but is known to irritate the eyes and cadmium can be contracted through the eyes. The dose level used in this study has been shown to be appropriate and tolerated by rabbits. Palm oil is a rich source of β-carotene which is the precursor of vitamin A, a known antioxidant (Gaydou *et al*., 1982). Palm oil is a common dietary component in the average meal of the people in southern Nigeria. This report provides data on the effect of palm oil on autooxidation, antioxidant enzymes and ATPases in tissues of rabbits in cadmium toxicity.

This study presents data which shows that cadmium administered ocularly reduces the weight of rabbits. This effect of cadmium on weight had consistently been reported (Asabga *et al*., 2004; Eriyamremu *et al*., 2005). The induction of weight loss by cadmium had been related to its influence on nutrient digestion and availability (Eriyamremu *et al*., 2005; Mesonero *et al*., 1993).

We observed that the kidney then the liver concentrated more cadmium than the brain (Table 2). The liver and the kidney are the main sites of metallothionein production and metal retention (Klaassen *et al*., 1999; Liu *et al*., 2000). It is therefore not surprising that cadmium level in these tissues were more than that in the brain. So one of the main reasons attributed to the high levels of cadmium in these organs is their capacity to

produce metallothionein which is believed to influence the uptake, distribution and toxicity of cadmium. The relatively low level of cadmium in the brain may be the result of the inability of the metal to effectively cross the blood brain barrier. Earlier studies by Crowe and Morgan (1997) had shown that this heavy metal is unable to cross this barrier effectively. So despite that the cadmium was administered through the eyes which have a close relationship with the optic nerve and then brain, the metal accumulates more in the liver and kidney than in the brain most probably due to this barrier. There was also a consistently raised level of MDA (a marker of oxidative damage to lipid-rich membranes) in the cadmium treated rabbits (Table 2). This is not surprising since cadmium is known to cause increase in lipid peroxidation (Gupta *et al*., 1991; Asabga *et al*., 2004). An increase in MDA in the tissues of the rabbits treated with cadmium would have profound effect as it is indicative of increased chances of tissue damage.

As a defense against these reactive free radicals, the body produces antioxidant enzymes like SOD and catalase which helps to mop them up. The observed decreases in SOD and catalase in the brain, liver and kidney issues of the cadmium treated rabbits (Table 3) is also consistent with previous reports (Gupta *et al*., 1991; Bagchi *et al*., 1996; Shaikh *et al*., 1999). This inhibition of these antioxidant enzymes will make such tissues prone to damage by free radicals and in part would have contributed to the observed increase in MDA of these tissues. Superoxide dismutase is a metalloenzyme requiring zinc for its structural stability and copper for its enzymatic activity (Gotz *et al*., 1994). Cadmium can also induce inhibition of this enzyme by binding to and displacing these metals from the active site of the enzymes (Gotz *et al*., 1994). This would have contributed to the observed reduction in SOD activity in the cadmium treated rabbits. The combined effect of increase in MDA and a reduction in SOD and catalase would elaborate tissue damage. As palm oil was shown to reduce MDA production and increase SOD and catalase, it would spare these tissues from damage. This effect of palm oil may be related to the ability of β-carotene to quench free radicals and prevent tissue damage (Kennedy and Liebler, 1992; Bendich, 1993). The ability of β-carotene to quench free radicals is also related to its concentration (Knekt *et al*., 1992) in the diet, it is not surprising therefore that in the animals fed 10% palm oil, SOD was restored to levels comparable with the control (Table 3). Also the levels of non-enzymatic antioxidants present in the palm oil and indeed the entire diet was not measured, however it is envisaged that they may contribute to free radical mop up.

ATPases are membrane bound enzymes whose activities will be affected with a disruption of membrane integrity. It is thus highly probable that the observed decrease in the activities of Na^+/K^+ ATPase, Mg^{2+} ATPase and Ca^{2+} ATPase in the liver and kidney of the rabbits administered with cadmium was due to the disruption of membrane integrity occasioned by free radicals. Ca^{2+} ATPase activity in the brain did not decrease (Table 4), so the effect of cadmium-induced free radical generation and its possible effects on membrane disruption, as the reason for decrease in the activities of these enzymes may not be over emphasized. It is acknowledged however that the major route of cadmium is liver to kidney. This becomes pertinent since the brain accumulated the least cadmium (Table 2). Again palm oil is rich in β-carotene which scavenges free radicals and spare tissues from damage thus implying that it would maintain the cell membrane integrity of the tissues. β-Carotene being lipid soluble would readily permeate the blood brain barrier and be available to the brain where it confers its protective effect. This may in part account for the restoration of brain ATPases to levels comparable to those of the control.

ATPases require energy (ATP) to function and ATP production in the brain, liver and kidney is mostly via oxidative phosphorylation that is closely associated with Mg^{2+} ATPase. In this study, we observed that exposure to cadmium decreased Mg^{2+} ATPase activity (Table 4). If indeed the decrease in Mg^{2+} ATPase observed in the cadmium exposed rabbits also reflects compromise in energy production, then it is not surprising that there were also decreases in the other ATPases particularly in the liver and kidney. It has been observed that in disease state, there is de-regulation of cell-type-specific programmes that control mitochondria biogenesis and function in different mammalian tissues (Scarpulla, 2002). So, the compromise in Mg^{2+} ATPase of the liver and kidney may thus have molecular relationships and may be early contributory biochemical event in the development of disease associated with cadmium. As palm oil reverses these effects on the enzyme, its components may have protective effects against cadmium toxicity.

Ca^{2+} ATPase is important in the transfer of Ca^{2+} ions that is known to mediate diverse array of physiological processes including gene expression and regulation (Hardingham *et al*., 1997). A decrease in liver and kidney Ca^{2+} ATPase activity in the cadmium administered rabbits (Table 4) would thus have profound consequences. Thus apart from the cadmium effect on lipid peroxidation, its possible effect on cell Ca^{2+} ions of the tissues would accelerate tissue damage. Palm oil feeding actually raised the level of Ca^{2+} ATPase activity and may thus increase Ca^{2+} ion transport which in turn may ensure the availability of the ion to mediate the processes that would protect the tissue from possible cadmium-induced damage. This would also hold for the liver and kidney, where the oil raised the activity of the ATPase.

In conclusion, whilst it is accepted that cadmium causes tissue damage by membrane lipid peroxidation due to its ability to generate free radicals and inhibit antioxidant enzyme, palm oil may offer some protection to brain, liver and kidney in cadmium toxicity by increasing SOD and ATPases activities.

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