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Protective effect of pre-supplementation with selenium on cadmium-induced oxidative damage to some rat tissues

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ABSTRACT

The effect of the antioxidant nutrient selenium, Se, on biologic response to cadmium-induced oxidative cytotoxicity was investigated in rats pretreated with Se prior to exposure to mild doses of Cd. Male wistar strain rats (200-250 g b.wt) were exposed to a single daily oral dose of cadmium (3 mg $CdCl_2$/kg) in drinking water for five days following a 15-day oral supplementation with Se (3.0 or 3.5 mg SeO_2/kg/day) in drinking water at the end of which membrane lipid peroxidation, ascorbic acid and glutathione contents and activities of antioxidant enzymes catalase, superoxide dismutase, and glutathione peroxidase were determined in the red blood cell, liver and kidney samples obtained from treated animals. Cadmium content was determined in liver and kidney samples. Results showed that pre-supplementation with Se effectively countered Cd-induced membrane lipid peroxidation, depletion of non-enzymic antioxidants, ascorbic acid, and glutathione, and induction of antioxidant enzymes catalase, superoxide dismutase, and glutathione peroxidase in the RBC, liver and kidney as well as reduced Cd accumulation in the liver and kidney, all of which effects are consistent with a protective effect of Se against Cd-induced oxidative cytotoxic damage. RBC was the most susceptible to the inhibitory effect of Se pre-supplementation on Cd-induced lipid peroxidation and tissue ascorbic acid and glutathione depletion.

Keywords: Cadmium, selenium, lipid peroxidation, antioxidant enzymes, non-enzymes endogenous antioxidants.

INTRODUCTION

Cadmium, Cd, a toxic heavy metal, is a ubiquitous industrial and environmental pollutant (Gonick, 2008), arising primarily from battery, electroplating, plastics, pigment, fertilizer industries and cigarette smoke. Humans become exposed to Cd pollutants through the food chain and the air. Following oral exposure, Cd is absorbed and delivered to the liver by endogenous intestinal protein metallothionein and from the liver it is rapidly redistributed to other organs with the kidney as the main target organ for Cd toxicity (Asagba, 2009). Public health interest in the toxic effects of environmental Cd was awakened by epidemiological evidence linking industrial Cd waste pollution of marine food sources to the outbreak of *itai itai disease* in Japan, a disease characterized by, among others, severe bone disorders and renal tubular lesions (Tomohito et al., 2010).

Studies with experimental animals have shown that exposure to Cd results in toxic lesions in many species, with Cd showing various mechanisms of toxicity in particular species under different experimental conditions (Waisberg et al., 2003). Severity of Cd intoxication of target organs is dependent on the route, dose, and duration of exposure and it manifests in various forms ranging from acute toxicosis to cancer. Cadmium causes toxic lesions in target organs by inducing oxidative stress (Patra et al., 2011). It has been demonstrated that Cd induces oxidative stress by stimulating the production of free radicals such as hydroxyl radicals (Patra et al., 2011), superoxide anions, nitric oxide and hydrogen peroxide (Waisberg et al., 2003), resulting in membrane lipid peroxidation and oxidative deterioration of proteins and DNA and in the process initiating various pathological conditions in humans and animals (Waisberg et al., 2003). Thus, a number of antioxidants and antioxidant defence systems have been shown to protect cells of target organs from Cd toxicity (Swaran, 2009) or to reverse Cd toxicity (Tandon et al., 2003).

Selenium, Se, an antioxidant micronutrient (Hatfield et al., 2009; Toman et al., 2009), that is an essential component of the antioxidant enzyme glutathione peroxidase and of a few other enzymes, has also been reported to exert certain protective effects against Cd toxicity to such target organs as the liver (Ognjanovic et al., 1995, 2008), the heart and kidneys (Ognjanovic et al., 2008). The reported protective roles include capability of Se to alter the distribution of Cd in tissues and to induces binding of the Cd–Se complexes to proteins which are similar to metallothioneins (Ognjanovic et al., 2008; Toman et al., 2009) and amelioration of Cd-induced oxidative stress and attendant oxidative damage (Ognjanovic et al., 2008). However, these reports on Se protective action against oxidative stress mediated Cd toxicity were from studies involving concurrent chronic exposure to Se and Cd while other possible forms of exposure appear not to have been appraised. Also the Se protective effect against Cd oxidative damage appear not to have been reported for the red blood cell, a tissue cell known to be susceptible to Cd-induced oxidative damage (Patra et al, 2011) and for it considerable uptake of Se (Ognjanovic et al., 2008). Thus, in this study we have examined the effect of pre-supplementation with Se on short term exposure to Cd at a comparatively mild dose-an appraisal of a condition where an organism already well nourished with Se becomes exposed to Cd.

We report on the protective effect of the micronutrient Se on Cd-induced oxidative lesions as determined by level of the pattern of tissue Cd bioaccumulation, membrane lipid peroxidation, and activity of the antioxidant defence system in the erythrocyte, hepatic and renal tissues of the rat that had been on Se supplementation.

MATERIALS AND METHODS
Animal treatment

Wistar Strain male rats (b.wt. 230-250 g) obtained from the Animal House Unit, University of Jos, were used in the study. They were maintained on a standard rat diet, 'Vital Feed' (purchased from Grand Cereals and Oil Mills Ltd, Kuru, Nigeria) and tap water as drinking water, *ad libitum*. The respective working doses of Se (as SeO_2) and Cd ($CdCl_2$) administered orally to experimental animals in this study was first determined in a pilot study. The Cd dose used was the lowest in the graded concentration of the Cd salt tolerated by the rats with quantifiable tissue biochemical changes without fatality. On the other hand, the Se dose used was the concentration that produced significantly definite positive in the antioxidant defence system capability compared to control rats fed normal diet alone.

Rats were distributed evenly, 4 rats/cages into four standard plastic-metal rat

cages, labeled A-D, respectively. Rats in the four groups were fed the standard 'Vital feed' rat diet and drinking water *ad libitum.* However, each rat in groups C and D received once daily, an oral supplement of Se as an aqueous solution of SeO_2 administered by means of a needle-free Syringe: each rat in group C received an oral dose of 3.0 mg SeO_2/kg b.wt/day while those in group D each received 3.5 mg SeO_2/kg b.wt/day. The daily oral Se supplementation was carried out for 15 days. Thereafter, rats in group, B, C and D were each given one single oral dose $CdCl_2$ in aqueous solution (3 mg $CdCl_2$/kg b.wt) daily for five days.

Tissue collection and preparation

At the end of the feeding experiment, on day 21, blood sample was collected from each rat in each group by cardiac puncture under chloroform anaesthesia using heparinized syringe. Each blood sample was transferred into a heparinized centrifuge tube and subsequently centrifuged at 3,000 r.p.m for 10 min on a MSE centrifuge to obtain red blood cells. The packed RBCs, in each case, were washed twice with ice-cold normal saline and then stored at 2 °C /kept on an ice bath pending biochemical analysis which was within a few hours.

After blood collection, rats still under anaesthesia were sacrificed by decapitation and, in each case, the liver and kidneys were excised and washed in ice-cold normal saline to remove adhering blood particles. Homogenates of liver and kidney samples of each rat were prepared separately by homogenizing 1 g portion in ice-cold 50 mM Tris-HCl buffer, pH7.4 (1:10, w/v) in an Akia homogenizer. The homogenates were centrifuged at 2,400 xg for 10 min in a refrigerated low-speed centrifuge and the supernatant (S1) fractions were collected with Pasteur Pipette into plastic vials and stored at 2 °C pending biochemical analysis. The rest of the kidney and liver samples were used for determination of Cd content.

Biochemical analysis

Membrane lipid peroxidation, non-enzymic tissue antioxidants (ascorbic acid and glutathione) concentration and antioxidant enzymes (superoxide dismutase, glutathione peroxidase and catalase) activity were determined in the liver and kidney supernatant fractions and red blood cells. Lipid peroxidation was estimated by the thiobarbituric acid reaction method described by Ohkawa et al. (1979). Ascorbic acid content was determined by the method of Roe and Kuether (1943) as modified by Tiez (1970) and glutathione concentration by the Ellman reaction (Ellman, 1959) method as described by Beutler et al. (1963). Catalase activity was assayed by the spectrophotometric method of Aebi et al. (1995); the assay of glutathione peroxidase activity based on the principle of oxidation of GSH to GSSG was by the procedure described by Egbuna (1992). Superoxide dismutase activity was assayed as described by Heikkila and Cabbat (1976).

Tissue cadmium determination

The cadmium contents of the liver and kidney tissue were estimated with inductively couple plasma optical emission spectrophotometer (ICP OES) optima 2000DV after wet digestion. 1g portion of the tissue was digested with 20 ml HNO_3-$HCLO_4$ mixture (1:4 v/v) at 100 °C and the resultant digest diluted to 100 ml with deionized water (Asagba et al., 2004).

Statistical analysis

Statistical analysis of numerical data (expressed as mean ± SD) was done using the statistical package for the social sciences software (SPSS) programme. One way analysis of variance (ANOVA) with post hoc analysis was used to assess the differences between the experimental groups and statistical significance was considered at $p<0.05$. In addition to ANOVA, the student's t-test was used to compare the various means.

RESULTS

Tissue cadmium content

The results of tissue cadmium determination are summarized on Table 1. As could be seen from the table, cadmium was detected in both the liver and kidney of control rats, suggesting that the experimental rats had previously been exposed to traces of Cd. The table shows that tissue Cd content was significantly higher in the liver and kidney of rats fed the normal diet alone, prior to exposure to cadmium (group B) than in the corresponding tissues of rats given the normal diet and 3 mg Se/kg prior to Cd exposure (group C). The tissue Cd contents of group B rats were also generally higher than those of rats given the normal diet and 3.5 mg Se/kg prior to exposure to Cd (group D) but the difference was significant only in the case of the kidney. This would suggest that prior supplementation with Se impaired Cd bioaccumulation in the liver and kidney in a dose dependent manner, with the lower Se dose being the more potent. The cadmium content of the kidney was generally high than that of the liver.

Membrane lipid peroxidation

Table 2 summarizes the results of the assay of membrane lipid peroxidation. The tissue concentrations of lipid peroxidation products (determined as malonaldehyde) are significantly higher in the RBC, liver and kidney if rats fed the normal diet prior to Cd exposure (group B) than in the corresponding tissues of the control rat (group A), suggesting that exposure to Cd stimulated membrane lipid peroxidation. Tissue concentrations of membrane lipid peroxidation products were also significantly higher in the RBC, liver and kidney of rats in group B than in the corresponding tissues of rats in either of the two groups pre-supplemented with Se, an indication that pre-supplementation with Se protected against Cd-induced lipid peroxidation. However, the level of lipid peroxidation was generally higher in the tissues of rats pre-supplemented with the higher Se dose (group D) than in those that received the lower Se dose (group C), suggesting that the protective effect of Se against Cd-induced membrane lipid peroxidation was dose-dependent, decreasing with increase in dose.

Non-enzymic tissue antioxidants

The results of tissue ascorbic acid and glutathione determinations are summarized on Tables 3 and 4, respectively. In each case, the levels of antioxidants were significantly much lower in the RBC, liver, and kidney of rats fed the normal diet prior to exposure to Cd (group B) than in the corresponding tissue of the control (group A), suggesting that exposure to Cd markedly depleted ascorbic acid and glutathione stores of the RBC, liver and kidney. Also, in each case, the levels of antioxidant were significantly lower in the RBC, liver and kidney of rats in group B than in the corresponding tissues of rats fed the normal diet supplemented with the lower Se dose prior to Cd exposure (group C). The difference in tissue antioxidant level between rats in group B and those fed the normal diet supplemented with the higher Se dose prior to Cd exposure (group D) was significant only in the case of the RBC. These would suggest that pre-supplementation with Se had a sparing effect on tissue ascorbic acid and glutathione, inhibiting their depletion by Cd; that the sparing effect is dose dependent with the lower Se being the more potent, and that the RBC was the most sensitive of the three tissue to the tissue antioxidant sparing effect of Se. Tables 3 and 4 also show that ascorbic acid and glutathione contents were highest, per unit tissue weight, in the RBC.

Antioxidant enzymes

Tables 5, 6 and 7 summarize the results of the assay of activities of tissue antioxidant enzymes catalase, superoxide dismutase and

glutathione peroxidase, respectively. Catalase activity (Table 5) is significantly higher in the RBC, liver and kidney of rats fed the normal diet prior to Cd exposure (group B) than in the corresponding tissues of the control, (group A), suggesting Cd induction of antioxidant enzyme activity. Also, catalase activity was significantly higher in the RBC, liver and kidney of rats in group B than in the corresponding tissues of rats fed the normal diet supplemented with the lower Se dose (group C); the difference in tissue catalase activity between rats in group B and those fed the normal diet supplemented with the higher Se dose (group D) is significant only in the case of the RBC. These would suggest that pre-supplementation with Se impaired Cd

induction of catalase activity in a dose-dependent manner, with the lower dose being the more potent. A similar trend was observed in the superoxide dismutase (SOD) and glutathione peroxidase (GPx) data except that activities of the two antioxidant enzymes were significantly higher in the RBC, liver and kidney of rats in group B than in the corresponding tissues of rats in the two groups fed the normal diet supplemented with Se prior to Cd exposure (groups C and D), suggesting that Se effectively antagonized Cd induction of tissue SOD and GPx activity at the two levels of Se pre-supplementation, although the lower Se dose remains the more potent.

Table 1: Effect of pre-supplementation with selenium on cadmium content of rat liver and kidneys.

Group	Treatment	Cadmium concentration (µg/ g tissue)	
		Liver	Kidney
A	ND (Control)	0.30±0.06	0.43±0.02
B	ND15days; then Cd	3.78±0.51[a]	4.72±0.72[a]
C	ND +Se(3mg/kg)15days,then Cd	2.39±0.53[ab]	2.83±0.45[ab]
D	ND +Se(3.5mg/kg)15days,then Cd	3.50±0.23[a]	3.42±0.21[ab]

Data are expressed as mean ± SD, n= 4 for each group. ND = Normal diet
[a] values are significantly different from control (p<0.05)
[b] values are significantly different from the group treated with Cd alone (p<0.05)

Table 2: Effect of pre-supplementation with selenium on cadmium- induced lipid peroxidation in RBC, liver and kidneys.

Group	Treatment	Malondialdehyde concentration (nmol/g tissue)		
		RBC	Liver	Kidneys
A	ND (Control)	5.41±0.79	14.95±0.59	19.66±0.79
B	ND 15days,then Cd	11.73±1.53[a]	40.25±0.88[a]	41.17±0.59[a]
C	ND +Se(3mg/kg)15days, Cd	6.21±1.02[ab]	28.98±0.84[ab]	25.07±0.96[ab]
D	ND +Se(3.5mg/kg)15days,Cd	8.33±1.21[ab]	35.77±1.42[ab]	29.67±2.33[ab]

Values are expressed as mean ± SD, n= 4 for each group.
[a] values are significantly different from control (p<0.05)
[b] values are significantly different from the group treated with Cd alone (p<0.05)
ND = Normal diet

Table 3: Effect of pre-supplementation with selenium on ascorbic acid levels in the RBC, liver and kidney of rats exposed to Cd.

Group	Treatment	Ascorbic acid concentration (mg/g tissue)		
		RBC	Liver	Kidneys
A	ND (Control)	1.57±0.03	1.34±0.04	1.13±0.03
B	ND 15days,then Cd	0.52±0.02[a]	0.71±0.04[a]	0.53±0.06[a]
C	ND +Se(3mg/kg)15days,Cd	1.06±0.02[ab]	1.01±0.11[ab]	0.93±0.06[ab]
D	ND +Se(3.5mg/kg)15days,Cd	0.91±0.09[ab]	0.73±0.04[a]	0.63±0.03[a]

Values are expressed as mean ± SD, n= 4 for each group. ND = Normal diet
[a] values are significantly different from control ($p<0.05$)
[b] values are significantly different from the group treated with Cd alone ($p<0.05$)

Table 4: Effect of pre-supplementation with selenium on tissue glutathione content of rats exposed to Cd.

Group	Treatment	Glutathione concentration (µmol/g tissue)		
		RBC	Liver	Kidneys
A	ND (Control)	0.095±0.001	0.090±0.002	0.071±0.001
B	ND 15days,then Cd	0.041±0.007[a]	0.034±0.002[a]	0.028±0.002[a]
C	ND +Se(3mg/kg)15days,Cd	0.061±0.009[ab]	0.062±0.002[ab]	0.052±0.004[ab]
D	ND +Se(3.5mg/kg)15days,Cd	0.056±0.007[ab]	0.054±0.003[a]	0.030±0.002[a]

Values are expressed as mean ± SD, n= 4 for each group. ND = Normal diet
[a] values are significantly different from control ($p<0.05$).
[b] values are significantly different from the group treated with Cd alone ($p<0.05$).

Table 5: Effect of pre-supplementation with selenium on catalase activity in tissues of rats exposed to Cd.

Group	Treatment	Catalase activity (µmol/g tissue)		
		RBC	Liver	Kidneys
A	ND (Control)	6.71±1.22	5.45±0.29	4.19±0.39
B	ND 15days, then Cd	16.57±1.81[a]	14.11±0.44[a]	11.18±1.02[a]
C	ND +Se(3mg/kg)15days,Cd	8.58±1.30[ab]	9.27±0.88[ab]	9.46±0.39[ab]
D	ND +Se(3.5mg/kg)15days,Cd	11.51±1.60[ab]	13.47±3.42[a]	11.17±0.69[a]

Values are expressed as mean ± SD, n= 4 for each group. ND = Normal diet
[a] values are significantly different from control ($p<0.05$)
[b] values are significantly different from the group treated with Cd alone ($p<0.05$)

Table 6: Effect of pre-supplementation with selenium on superoxide dismutase activity in tissues of rats exposed to Cd.

Group	Treatment	SOD activity (unit/g tissue)		
		RBC	Liver	Kidneys
A	ND (Control)	2.78±3.2	20.22±12.71	6.86±5.88
B	ND 15days,then Cd	97.22±3.21[a]	85.11±3.47[a]	67.54±2.03[a]
C	ND+Se(3mg/kg)15days,Cd	41.66±3.2[ab]	27.66±2.45[ab]	41.18±2.63[ab]
D	ND+Se(3.5mg/kg)15days,Cd	66.67±4.53[ab]	43.62±2.12[ab]	65.68±1.96[a]

Data are expressed as mean ±SD, n= 4 for each group.
[a] values are significantly different from control ($p < 0.05$)
[b] values are significantly different from the group treated with Cd alone ($p < 0.05$)
ND = Normal diet

Table7: Effect of pre-supplementation with selenium on glutathione peroxidase activity in tissues of rats exposed to Cd.

Group	Treatment Group	GPx activity (unit/g tissue)		
		RBC	Liver	Kidneys
A	ND (Control)	52.45±5.35	63.47±3.49	62.80±2.59
B	ND 15days, then Cd	98.88±1.97[a]	139.10±3.48[a]	139.10±3.49[a]
C	ND+Se(3mg/kg)15days,Cd	67.52±1.97[ab]	67.52±2.21[ab]	73.61±2.59[ab]
D	ND+Se(3.5mg/kg)15days,Cd	84.41±1.97[ab]	85.08±3.49[ab]	81.03±3.82[ab]

Values are expressed as mean ± SD, n= 4 for each group
[a] values are significantly different from control ($p < 0.05$)
[b] values are significantly different from the group treated with Cd alone ($p < 0.05$)
ND = Normal diet

DISCUSSION

Our study has examined the effect of selenium on the response of the antioxidant defence and cadmium disposition systems to exposure to mild concentrations of cadmium in a situation where the experimental animal has undergone Se supplementation prior to Cd exposure (that is, the logical opposite of a situation where the experimental animal was made Se deficient prior to Cd exposure). This approach is at variant with recent reports which appear to focus essentially on the response of these systems in situations where Cd and Se were administered concurrently to the experimental animal or where Cd intoxicated organism is fed Se.

On cadmium disposition, our finding is that prior supplementation with selenium significantly alters the pattern of Cd disposition, markedly reducing the level of bioaccumulation of Cd in the rat liver and kidney. This behaviour is consistent with a protective effect of selenium from Cd toxicity to these target organs. No such significant reduction in Cd bioaccumulation was observed in a study on rats in which Cd and Se were given concurrently for a much period and at a much high Cd dose (Ognjanovic et al., 2008) notwithstanding that the overall data reflected a protective effect for Se.

The absorption of cadmium from the gut lumen is mediated by an endogenons intestinal protein metallothionein (Asagba, 2009) which also facilitates Cd transport to the liver and presumably the redistribution of the metal ion to the kidney and other organs of

the body. Cadmium and selenium have very closely related absorption and transport mechanisms and metallothionein is reportedly a key factor in the protective effects of Se from Cd toxicity. Selenium transport proteins are so very closely similar to cadmium binding metallothionein that they can bind both metal ions (Toman et al., 2009) and selenium has been shown to hold the key to the binding of Se-Cd complexes to those of metallothionein-like proteins. It would therefore appear that following pre-supplementation with Se over a 15 day period prior to Cd exposure, the mutual transport system may have become primed for Se absorption and transport, thereby reducing the rate of Cd absorption and bioaccumulation in the rat liver and kidney, a development consistent with a protective role for selenium in cadmium hepatotoxicity and renal toxicity. This would appear to also explain why no reduction in Cd bioaccumulation in the liver and kidney was observed following concurrent chronic exposure to Cd and Se.

The generally higher concentration of Cd in the kidney than in the liver is consistent with its being the major target organ for cadmium toxicity (Asagba, 2009). The presence, albeit in trace quantities, of cadmium in the liver and kidney of rats on the control diet has also been observed by other workers (Asagba et al., 2004; 2006; Ognjanovic et al., 2008). It is a testimony of the ubiquity of cadmium in the food chain and the environment; including the plastic components of the rat cage. The several fold lower tissue concentrations of Cd observed in this study in rats exposed to Cd compared to those reported by other workers (Asagba et al., 2004; 2006; Ognjanovic et al., 2008) are a reflection of the relatively mild dose and short duration of Cd exposure. These workers exposed rats to Cd at the levels of 100 mg Cd/kg for 16 weeks (Asagba et al., 2004; 2006) and 15 mg Cd/kg for 4 weeks (Ognjanovic et al., 2008), respectively compared to our 3 mg/Cd/kg for 5 days.

Membrane lipid peroxidation is a major early manifestation of oxidative damage to the liver and kidney tissues by cadmium (Swaran, 2009) and it plays on important role in the induction of toxic lesions by many pro-oxidant foreign metal ions like Cd (Waisberg et al., 2003). Our findings confirm that exposure to cadmium causes a significant increase in membrane lipid peroxidation in the liver, kidney and in the red blood cells. Our finding that prior supplementation with Se significantly reduced the level of Cd-induced membrane lipid peroxidation in the three tissues is consistent with a protective effect of Se from Cd toxicity to the rat and is in accord with reported protective effects of antioxidant nutrients against Cd-induced oxidative stress and lipid peroxidation in the liver and kidney (Deepti et al., 2010). A similar reduction in the level of Cd-induced lipid peroxidation in the liver and kidney has been reported in rats fed Se and Cd concurrently. The extent of reduction of Cd-induced lipid peroxidation by Se pre-supplementation (3 mg Se/kg) was highest in the RBC (47.06%)followed by the kidney (39.11%) and liver (28.0%) suggesting that Se mitigation of cadmium-induced lipid peroxidation is most effective in the RBC.

The protective role of endogenous non-enzymic antioxidants against oxidative damage by free oxy-radicals and the depletion of their tissue reserves in the process have long been documented (Swaran, 2009). They are used up in the course of destroying the oxy-radicals. Our study found that exposure to cadmium induced a significant depletion of ascorbic acid and glutathione in the rat RBC, liver and kidney, a lesion which was effectively prevented by pre-supplementation with Se. Depletion of tissue reserves of endogenous antioxidants, ascorbic acid and glutathione, on exposure to Cd is the expected biologic response since cadmium is known to induce oxidative stress through generation of oxy- radicals (Swaran, 2009). That exposure to cadmium induces depletion of ascorbic acid in the blood, liver and kidney is well documented (Patra et al., 2011). The observed

sparing effect of Se pre-supplementation on Cd-induced depletion of both antioxidants in the rat RBC, liver and kidney is consistent with a protective role for Se in Cd toxicity. The extent of the sparing of endogenous antioxidants from Cd induced depletion by selenium supplementation (3 mg Se/kg) was highest in the RBC (103.85%) followed by the kidney (43.01%) and liver (42.25%) in the case of ascorbic acid, but the reverse was the case with glutathione the kidney (85.71%), liver (82.35%) and RBC (48.78%). This would appear to suggest that sparing effect of Se pre-supplementation or tissue ascorbic acid and glutathione was most effective in the RBC and kidney, respectively. The underlying mechanism of this tissue difference in Se action is not readily understood. No significant sparing effect was observed in the hepatic and renal ascorbic acid content of rats given Se and Cd concurrently (Ognjanovic et al., 2008) – the ascorbic acid depleting effect was more pronounced than the sparing effect of Se. As with Cd-induced depletion, there appears to be no report on the effect of Se on Cd-induced glutathione depletion in the three tissues.

Catalase, superoxide dismutase (SOD), and glutathione peroxidase are the three primary antioxidant enzymes of the endogenous defence systems for the removal of reactive oxygen species (Patra et al., 2011), being the first enzymatic antioxidant defences for the body against oxidant-induced cytotoxic challenge. Upon ingestion, many xenobiotics, including proxidants, such as 2, 4-dinitrophenyl hydrazine (Maduka and Okoye, 2002), induce the enzymes that metabolize them. 2, 4-Dinitrophenyl hydrazine causes lipid peroxidation in the body through the formation of superxide anions. Our results have shown that in all three cases, exposure to cadmium significantly induced enzyme activity which was effectively reversed by pre-treatment with selenium. However, these findings are at variance with results obtained from studies on long-term exposure to relatively high doses of cadmium which

observed Cd inhibition of SOD in the rat liver and kidney (Asagba et al., 2004; Ognjavic et al., 2008) and RBC and of rat liver and kidney glutathione peroxidase and liver catalase (Ognjanovic et al., 2008). The inhibitory action of Cd on the three antioxidant enzymes was reportedly effectively reversed on concurrent exposure to both Cd and Se (Ognjanovic et al., 2008). The apparent contradiction between these reports and our present findings is traceable to the difference in the oral dose of Cd and duration of exposure. As discussed earlier, our cadmium dose (3 mg/kg) is relatively mild compared to the working Cd dose of between 15 mg Cd/kg (Ognjanovic et al., 2008) and 100 mg Cd/kg (Asagba et al., 2004) employed by these other workers for durations ranging from four weeks (Ognjanovic et al., 2008) to 16 weeks (Asagba et al., 2004) to the five-day Cd exposure in our study. These differences are reflected in the tissue load of cadmium: the mean liver and kidney Cd content of 3.78 ± 0.51 and 4.72 ± 0.72 mg Cd/g tissue respectively, obtained in our study are extremely mild compared to the corresponding values of 21.26 ± 1.72 and 23.4 ± 1.68 mg/g tissue (Ognjanovic et al., 2008) and 50.50 ± 1.43 and 75.30 ± 0.94 mg Cd/g tissue (Asagba et al., 2004) reported by other workers. At such high tissue concentrations, Cd is believed to exert direct toxic effects on the antioxidant enzymes (Ognjanovic et al., 2008), including inhibition of enzyme activity through displacement of divalent cations from the enzyme active site of the respective antioxidant enzymes; which are metalloenzymes.

One common trend observed in the results discussed above was the dose dependence of Se influence on Cd action on the rat tissue. The extent of Se moderation of Cd action was such that while the influence of the lower dose of selenium (3 mg Se/kg) on Cd-induced lesions invariably significantly affected all the three tissues, RBC, liver and kidney, that of the higher dose (3.5 mg Se/kg) with the exception of lipid peroxidation and

SOD and glutathione peroxidase, significantly affects only one or two tissues: impairment of Cd accumulation only in the liver; sparing effect on ascorbic acid and glutathione only in the RBC and induction of catalase only in the liver. These would suggest that the dose used in this study may be in the upper segment of the three hold of Se dose that influence Cd action. Ognjanovic and coworkers (2008) administered Se at a much lower dose (0.5 mg Se/kg) that was employed in this study.

Conclusion

It can be concluded from the findings of our investigations as discussed above that Se sufficiency offers protection against Cd challenge in particular, that an organism well nourished with respect to selenium at the time of Cd exposure has a more effective defence capability against Cd-induced oxidative damage to tissue than otherwise as determined by the various parameters. The extent of influence of prior supplementation with Se on the Cd disposition system, membrane lipid peroxidation and the antioxidant defence system are consistent with a protective influence of Se on Cd induced oxidative stress and cytotoxicity.

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