

Full Length Research Paper

# The protective role of *Scoparia dulcis* on tissue antioxidant defense system of rats exposed to cadmium

M. A. Adaikpoh<sup>1</sup>, N.E.J Orhue<sup>1\*</sup> and I. Igbe<sup>2</sup>

<sup>1</sup>Department of Biochemistry, Faculty of Life Science, University of Benin, P.M.B. 1154, Benin City, Nigeria.

<sup>2</sup>Department of Pharmacology, Faculty of Pharmacy, University of Benin, P.M.B.1154, Benin City, Nigeria.

Accepted 27 June, 2006

The possible antioxidant property of aqueous extract of *Scoparia dulcis* was tested in rats exposed to cadmium. Different groups of animals were treated with CdCl<sub>2</sub> alone or in combination with graded levels of *S. dulcis* (i.e. 250, 500 and 1000 mg/kg body wt, respectively). Cadmium, a known prooxidant was administered subcutaneously (3 mg /kg body wt), once every week, for two weeks. The required dose of aqueous extract of *S. dulcis* was administered daily for 2 weeks by gavage. The results show that relative to controls, cadmium significantly ( $P<0.05$ ) reduced superoxide dismutase activity while significantly ( $P<0.05$ ) increasing catalase activity and malondialdehyde levels in the liver and kidney. However, no significant effect was observed in the antioxidant enzyme activities and MDA levels in the heart. The dose of 1000 mg *S. dulcis*/kg body wt, like cadmium when administered alone, exhibited a prooxidant effect but when coadministered with cadmium, the high dose of *S. dulcis* effectively restored the antioxidant enzyme activities in the kidney and liver to levels that are not statistically different from the control. These observations show that aqueous extract of *S. dulcis* possesses significant antioxidant activity, sufficient to mitigate against free radical induced oxidative stress in experimental cadmium intoxication in the rat.

**Key words:** Cadmium, *Scoparia dulcis*, antioxidant enzymes, malondialdehyde.

## INTRODUCTION

Cadmium is a well known environmental toxin. Though it has no known biological function, it is widely used in the industry for electroplating and galvanization processes (Wilson, 1988). It is also useful in the production of pigments, batteries, glass and as components of many alloys (ATSDR, 1989). Effluents from these industries often pollute water, air and food with this metal. Some cadmium has been found in all natural materials that have been analyzed (Massanyi et al., 2000). The kidney and the liver are the major target organs of cadmium accumulation and intoxication (WHO, 1992). Exposure to cadmium leads to renal tubular dysfunction. This is primarily expressed as a renal tubular reabsorption defect, and

is now recognized as a cardinal feature of cadmium induced renal damage (Mueller, 1993; Horiguchi et al., 1996). Cadmium is also implicated in the etiology of hypertension (Lall et al., 1997). The metal is currently believed to cause most of its toxic effects by mechanism(s) related to its ability to generate free radicals at a rate high enough to overwhelm the natural antioxidant defense system of the body (Bagchi et al., 1996).

Current strategies in the management of cadmium toxicity revolve around the use of chelating agents. The toxic effects arising from the use of these agents (Jones et al., 1988) has led to an upsurge in research into the use of nutrients and phytochemicals in the management of the toxic effects due to this metal.

*Scoparia dulcis* or sweet broom weed is fast becoming a medicinal plant of growing global interest. A number of

\*Corresponding authors E-mail: [jerryorhue@yahoo.com](mailto:jerryorhue@yahoo.com)

the speculated medicinal properties of *S. dulcis* have been validated by scientific research. These include hypoglycaemic activity (Pari and Venkateswaran, 2002) antioxidant activity (Pari and Latha, 2004), antitumour promoting activity (Nishino, 1993) and antiviral activity (Hayashi, 1990). Ratnasooriya et al. (2003) have also reported a significant analgesic and antihyperalgesic activity for *S. dulcis* decoction. Phytochemical screening of the herb revealed that it is rich in flavonoids and terpenes. It is believed that the pharmacological actions of *S. dulcis* are due to the presence of these phytochemicals (Hayashi, 1990, 1991; Kawasaki, 1987; Ahmed and Jakupovic, 1990).

The aim of this study is to investigate the effect of aqueous extract of *S. dulcis* on cadmium induced oxidative stress in the heart, liver and kidney of rats exposed to this heavy metal by measuring the extent of oxidative damage and the status of two antioxidant enzymes, catalase and super oxide dismutase.

## METHOD

### Animal feeding and treatment

Thirty male wistar strain rats (average weight 200 g), purchased from the laboratory animal unit of the Lagos University Teaching Hospital (LUTH), Lagos, Nigeria were used for this study. After two weeks adaptation to our laboratory conditions, animals were randomly assigned to six groups of five rats each.

Animals were housed in clear wire cages with mesh floor and allowed free access to food and water throughout the duration of the study. The first group which served as control (i.e. -Cd-SD) (SD=*S. dulcis*) was maintained on normal rat chow (Bendel Feeds and Flour Mill, Ewu, Nigeria). The second group received cadmium alone (i.e. +Cd-SD) while the third group was maintained on 1000 mg aqueous extract of *S. dulcis* /kg body weight (i.e. -Cd+SD<sub>1000</sub>). The other three groups (+Cd+SD<sub>250</sub>; +Cd+SD<sub>500</sub> and +Cd+SD<sub>1000</sub>) in addition to cadmium, also received graded doses of aqueous extract of *S. dulcis*, the subscript denoting the dose of S.D/Kg body weight/day. The aqueous extract of *S. dulcis* was administered daily by gavage while cadmium (3 mg CdCl<sub>2</sub>/kg body weight) or its vehicle (normal saline) was administered subcutaneously, once every week. The animals were weighed every week and accordingly, the dose of CdCl<sub>2</sub> was adjusted on weekly basis. The groups that were not treated with cadmium were given equivalent amounts of normal saline. The "Guiding Principles in the Use of Animals in Toxicology" was followed during the study.

At the end of the two weeks study period, animals were sacrificed under chloroform anesthesia. Fresh blood was collected in heparinized tubes for plasma preparation while the Heart, Kidney and Liver were excised, washed in ice cold normal saline to remove blood, and stored at -4°C until needed for biochemical analysis, which took place within 48 h.

### Preparation of *Scoparia dulcis* plant extract

Five hundred grams of air dried and pulverized shoot portions of the plant, *S. dulcis* was soaked in 1.5 L of distilled water overnight. This was subsequently filtered into a beaker using filter paper and funnel. The filtrate was concentrated at 40°C to constant weight using a rotavapor apparatus. The residue was collected and stored at -4°C. The concentrate was then reconstituted into a stock solution

of 200 mg/ml in distilled water. The required volume of this solution (calculated on the basis of animal weight) was administered daily by gavage.

### Preparation of tissue homogenate

Weighed portions of the tissues were homogenized in ice cold 0.05 M phosphate buffer pH 7.8 to obtain a 20% (w/v) homogenate as described by Aksnes and Njaa (1981). The homogenates were centrifuged at 10,000 g for 15 min and the clear supernatant obtained was immediately used for the analysis of antioxidant enzymes. For the assay of malondialdehyde, tissues were homogenized in normal saline. After centrifugation, the supernatant was used for the assay.

### Biochemical analysis

The concentration of malondialdehyde was assayed as thiobarbituric acid reactive substances (TBARS) in the tissues. TBARS is widely accepted indicator of lipid peroxidation. Malondialdehyde was determined according to the method of Buege and Aust (1998). Catalase activity was determined by the method of Cohen et al. (1970). Each catalase (CAT) unit specifies the relative logarithmic disappearance of hydrogen peroxide per minute and is expressed as K min<sup>-1</sup>, while superoxide dismutase (SOD) activity was estimated by the method of Misra and Fridovich (1972) and computed as described by Baum and Scandalios (1981). In this procedure, one unit represents the amount of the enzyme required for 50% inhibition of the conversion of epinephrine to adrenochrome during one minute.

### Statistical analysis

The results were expressed as means ± S.D. Statistical analysis was by one way Analysis of Variance (ANOVA). The differences between the means were tested by Turkey Kramer Multiple Range tests. Values of P<0.05 were considered statistically significant.

## RESULTS

Table 1 shows that cadmium significantly (P<0.05) decreased SOD activity and significantly (P<0.05) increased catalase activity and malondialdehyde (MDA) levels in the liver. However, in the presence of *S. dulcis*, there was a gradual improvement in the antioxidant status of the liver. The various parameters investigated approached control levels in a dose dependent fashion, with the ameliorating effect of the herbal extract increasing with increase in concentration of *S. dulcis* from 250 mg/kg body wt to 1000 mg/kg body wt. At 1000 mg *S. dulcis*/kg body wt, these parameters were each restored to levels that were not statistically different from the control.

The effect of cadmium and *S. dulcis* on kidney SOD, CAT and MDA are presented in Table 2. The data shows that cadmium inhibits SOD activity and *S. dulcis* removes this inhibitory effect in a dose dependent pattern. Also, the data shows that CAT activity was significantly (P<0.05) higher in all the test groups compared with the control.

**Table 1.** Changes in the liver malondialdehyde levels and antioxidant enzyme activities of rats exposed to cadmium and aqueous extracts of *Scoparia dulcis*.

Group	Superoxide dismutase activity (unit/g tissue) × 10 <sup>-3</sup>	Catalase activity (K/ min)	Malondialdehyde level (units/g tissue) × 10 <sup>-5</sup>
-Cd - SD	8.14 ± 0.58 <sup>a</sup>	0.89 ± 0.13 <sup>a</sup>	3.02 ± 0.47 <sup>a</sup>
+Cd - SD	3.66 ± 0.50 <sup>b</sup>	1.29 ± 0.05 <sup>b</sup>	5.58 ± 1.05 <sup>b</sup>
-Cd + SD <sub>1000</sub>	3.66 ± 0.53 <sup>b</sup>	1.32 ± 0.04 <sup>b</sup>	4.19 ± 0.51 <sup>b</sup>
+Cd + SD <sub>250</sub>	2.35 ± 0.49 <sup>b</sup>	0.95 ± 0.10 <sup>a</sup>	8.10 ± 0.24 <sup>a</sup>
+Cd + SD <sub>500</sub>	5.04 ± 0.68 <sup>b</sup>	1.07 ± 0.08 <sup>a</sup>	6.21 ± 0.37 <sup>b</sup>
+Cd + SD <sub>1000</sub>	10.38 ± 2.42 <sup>a</sup>	1.15 ± 0.11 <sup>b</sup>	4.08 ± 1.06 <sup>a</sup>

The results are means ± S.D. (n =5).

Values on the same column with different alphabets differ significantly (P<0.05).

-Cd-SD group was maintained on normal rat chow;

+Cd-SD group received only (3 mg CdCl<sub>2</sub>/kg body wt);

-Cd + SD<sub>1000</sub> group received only 1000 mg aqueous extract of *S. dulcis*/kg body wt.

+Cd + SD<sub>250</sub>; +Cd + SD<sub>500</sub> and +Cd + SD<sub>1000</sub> groups were maintained on 250, 500 and 1000 mg aqueous extract of *S. dulcis*/kg body wt and cadmium (3 mg CdCl<sub>2</sub>/kg body wt).

**Table 2.** Changes in the kidney malondialdehyde levels and antioxidant enzyme activities of rats exposed to cadmium and aqueous extracts of *Scoparia dulcis*.

Group	Superoxide dismutase activity (unit/g tissue) × 10 <sup>-3</sup>	Catalase activity (K/ min)	Malondialdehyde level (units/g tissue) × 10 <sup>-5</sup>
-Cd - SD	10.93 ± 2.25 <sup>a</sup>	0.90 ± 0.11 <sup>a</sup>	2.83 ± 0.42 <sup>a</sup>
+Cd - SD	6.00 ± 0.55 <sup>b</sup>	1.34 ± 0.05 <sup>b</sup>	6.05 ± 0.67 <sup>b</sup>
-Cd + SD <sub>1000</sub>	11.36 ± 0.69 <sup>a</sup>	1.39 ± 0.04 <sup>b</sup>	4.15 ± 0.11 <sup>a</sup>
+Cd + SD <sub>250</sub>	7.01 ± 0.40 <sup>b</sup>	1.24 ± 0.06 <sup>b</sup>	12.69 ± 1.15 <sup>b</sup>
+Cd + SD <sub>500</sub>	5.93 ± 1.13 <sup>b</sup>	1.26 ± 0.09 <sup>b</sup>	6.79 ± 1.28 <sup>b</sup>
+Cd + SD <sub>1000</sub>	11.17 ± 0.04 <sup>a</sup>	1.38 ± 0.11 <sup>b</sup>	5.30 ± 0.08 <sup>b</sup>

The results are means ± SD (n =5).

Values on the same column with different alphabets differ significantly (P<0.05)

-Cd-SD group was maintained on normal rat chow;

+Cd-SD group received only (3 mgCdCl<sub>2</sub> / kg body wt);

-Cd + SD<sub>1000</sub> group received only 1000 mg aqueous extract of *S. dulcis*/kg body wt.

+Cd + SD<sub>250</sub>; +Cd + SD<sub>500</sub> and +Cd + SD<sub>1000</sub> groups were maintained on 250, 500 and 1000 mg aqueous extract of *S. dulcis* /kg body wt and cadmium respectively.

The pattern shown by MDA in the kidney is similar to that shown in the liver. Although the MDA levels of all the cadmium and *S. dulcis* treated groups were significantly (P<0.05) higher than control, a gradual decrease was observed as the dose of *S. dulcis* increased. At *S. dulcis* 1000mg/kg body wt, MDA levels were not significantly (P>0.05) different from control.

The effect of cadmium and *S. dulcis* on the SOD and CAT activities as well as the MDA levels in the heart is different from the pattern observed in the kidney and liver (Table 3). Here, cadmium did not reduce SOD activity. All the cadmium and *S. dulcis* treated groups showed an increase in SOD activity but this increase was only significant (P < 0.05) at *S. dulcis* levels of 250 and 500 mg/kg body wt. Also the CAT activity in all the treated groups was not significant (P>0.05) when compared with the control. However, the MDA levels of the group treated with cadmium and 250 mg aqueous extract of *S.*

*dulcis*/kg body wt (i.e.+Cd+SD<sub>250</sub>) was significantly (P< 0.05) higher than control.

## DISCUSSION

This study examined the effect of aqueous extract of *S. dulcis* on changes that occur in the antioxidant enzyme system and the extent of oxidative damage in the heart, liver and kidney of rats exposed to cadmium. The results show that cadmium significantly decreased SOD activities in the liver and kidney but increased it, though not significantly, in the heart. Earlier studies (Gupta, et al., 1991; Stajn et al., 1997) have reported an inhibition of SOD activity in cadmium intoxication but others (Sarkar et al., 1995), reported an increased activity of the enzyme in the heart. This difference in the effect of the metal on tissue SOD activity may be attributed to differences in ca-

**Table 3.** Changes in the Heart malondialdehyde levels and antioxidant enzyme activities of rats exposed to cadmium and aqueous extracts of *Scoparia dulcis*.

Group	Super oxide dismutase Activity (unit/g tissue) × 10 <sup>-3</sup>	Catalase activity (K/ min)	Malondialdehyde Level (units/g tissue) × 10 <sup>-5</sup>
-Cd - SD	1.01 ± 0.11 <sup>a</sup>	1.70 ± 0.13 <sup>a</sup>	3.12 ± 0.21 <sup>a</sup>
+Cd - SD	2.10 ± 0.68 <sup>a</sup>	1.80 ± 0.04 <sup>a</sup>	3.47 ± 0.94 <sup>a</sup>
-Cd + SD <sub>1000</sub>	2.09 ± 0.06 <sup>a</sup>	1.71 ± 0.22 <sup>a</sup>	3.91 ± 0.27 <sup>a</sup>
+Cd + SD <sub>250</sub>	2.90 ± 0.16 <sup>b</sup>	1.79 ± 0.07 <sup>a</sup>	5.68 ± 1.32 <sup>b</sup>
+Cd + SD <sub>500</sub>	2.76 ± 0.67 <sup>b</sup>	1.75 ± 0.07 <sup>a</sup>	3.63 ± 0.14 <sup>a</sup>
+Cd + SD <sub>1000</sub>	1.31 ± 0.18 <sup>a</sup>	1.69 ± 0.10 <sup>a</sup>	4.00 ± 0.04 <sup>a</sup>

The results are means ± SD (n =5)

Values on the same column with different alphabets differ significantly (P<0.05)

-Cd-SD group was maintained on normal rat chow;

+Cd-SD group received only (3 mgCdCl<sub>2</sub> / kg body wt);

-Cd + SD<sub>1000</sub> group received only 1000 mg aqueous extract of *S. dulcis*/kg body wt.

+Cd + SD<sub>250</sub>; +Cd + SD<sub>500</sub> and +Cd + SD<sub>1000</sub> groups were maintained on 250, 500 and 1000 mg aqueous extract of *S. dulcis* /kg body wt and cadmium respectively

dmium load in these organs following the exposure to this metal. Massanyi et al. (1995) reported a very high level of cadmium in the liver following a single interperitoneal administration and a later redistribution to other tissues especially the kidney. This, therefore, makes the kidney the primary site of cadmium toxicity (Friedman and Gasek, 1994). Some of the toxic effects of cadmium are due to its inhibition of various enzyme systems. Cadmium is chemically similar to zinc and competes with it for inclusion in metalloenzymes, thus inactivating such enzymes (Vallee and Ulmer, 1972). Since SOD contains zinc, it is likely that cadmium could have inhibited the enzyme via this mechanism. It is interesting though, that in both the liver and kidney, *S. dulcis* at 1000 mg/kg body wt was able to restore the enzyme activity to control levels but at lower doses (i.e. 250 and 500mg/kg body wt), the herbal extract could not effectively remove the inhibitory effect of the metal on the enzyme. This shows that *S. dulcis* has a threshold level at which it becomes an efficient antioxidant when co-administered with molecules that cause oxidative stress.

Also our results show that treatment with cadmium increased lipid peroxidation in the tissues. This is similar to earlier reports (Bagchi et al., 1996; Sarkar et al., 1995). Treatment with high concentration of aqueous extract of *S. dulcis* (1000 mg/ kg body wt) was very effective in the prevention of oxidative damage induced by cadmium which resulted in lower malondialdehyde levels. The fact that treatment with *S. dulcis* extract increased the activity of the antioxidant enzymes and reduced tissue MDA levels suggests that the herb may help to control free radicals. *S. dulcis* has been reported to be rich in alkaloids and terpenoids (Pari and Latha, 2004; Loew and Kaszkin, 2002) which are well-known antioxidants, that can scavenge the free radicals.

The activity of the other antioxidant enzyme considered in this study, CAT was not altered in the heart but in the kidney and liver, the enzyme activity was significantly

higher than control. The increase in CAT activity may be the body's way of adjusting to the reduction in SOD activity.

It can be concluded from the data presented that administration of *S. dulcis* following experimental cadmium intoxication resulted in a dose dependent amelioration of the cadmium induced oxidative stress, as assessed by the levels of MDA and the activities of the antioxidant enzymes, catalase and SOD. However the most effective dose of *S. dulcis* (1000 mg/Kg body weight) when administered alone (i.e. in the absence of cadmium) behaved in much the same way as the heavy metal, potentiating oxidative damage. Although these observations are yet to be fully understood, it seems logical to suggest that *S. dulcis* effectively mitigates against the prooxidant activities of cadmium, and that very high doses of *S. dulcis* may be capable of causing oxidative damage.

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