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## Evaluation of the Effects of Aqueous Extracts of *Hibiscus sabdariffa* Calyces on Cadmium-Induced Oxidative Damage in Rats

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**Abstract:** The effects of aqueous extracts of *Hibiscus sabdariffa* L. calyces in cadmium-induced oxidative damage in serum and tissues of rats were evaluated in this study. Twenty eight male Wister rats (170-200 g) in four groups, {control (I), *Hibiscus sabdariffa* (II), Cadmium (III) and *H. sabdariffa*+Cd (IV)}, were used. Groups II and IV were given a daily dose of 0.2 g kg<sup>-1</sup> body weight of *H. sabdariffa* extracts for four weeks, while groups III and IV (the Cd groups) were injected sub-cutaneously with 0.002 g Cd (as CdSO<sub>4</sub>.8H<sub>2</sub>O) kg<sup>-1</sup> body weight once a week for four weeks. Liver protein levels significantly (p<0.05) decreased for all test groups relative to control, while the kidney and testis protein levels significantly (p<0.05) increased. There was a general increase in serum and tissue malondialdehyde (MDA) levels of tests groups relative to control while a general decrease was observed in serum and tissue catalase activities. Serum SOD of tests groups significantly (p<0.05) decreased while tissue SOD significantly (p<0.05) increased compared to control. The results showed, for some parameters evaluated, that *H. sabdariffa* appeared to play a protective role against Cd-induced oxidative damage. Paradoxically, however, *H. sabdariffa* alone also appeared to exert some measure of oxidative damage.

**Key words:** *Hibiscus sabdariffa* L., cadmium, oxidative damage, malondialdehyde, catalase, superoxide dismutase

### INTRODUCTION

Cadmium is a widespread environmental pollutant, present in food (mainly cereals, vegetables and shellfish) and tobacco. It poses a threat to human health because of its long retention (decades) in the kidneys (Järup *et al.*, 1998).

Cd does not appear to generate free radicals by itself, but it has been shown to produce hydroxyl radicals in the presence of metallothioneins. This suggests that cadmium-mediated production of reactive oxygen species (ROS) results from subsequent damage to critical organelles (e.g., mitochondria), displacement of endogenous redox active metals (e.g., Fe, Cu) or decrease of radical scavengers (e.g., Glutathione) (Stohs and Bagchi, 1995). Cadmium mediated oxidative stress also causes DNA strand breaks, lipid peroxidation and generation of oxidatively modified proteins (Stohs and Bagchi, 1995).

*Hibiscus sabdariffa* (Roselle) belongs to Malvaceae family. It is an erect, mostly branched, annual shrub. Flowers are red to yellow with a dark center containing short-peduncles (Qi *et al.*, 2005). The fresh calyces are

rich in riboflavin, ascorbic acid, niacin, carotene, calcium and iron that are nutritionally important (Qi *et al.*, 2005).

The calyces are used to make cold and hot beverages in many of the world's tropical and sub-tropical countries. The juices make a very colourful wine (Duke, 1983). In the United States, the Food and Drug regulation permits the use of the extracts in alcoholic beverages (Facciola, 1990). *H. sabdariffa* is found to contain antioxidants such as flavonoids, polyphenolics and anthocyanines. The presence of these chemicals in the flowers help to prevent the oxidation of low density lipoproteins (Bown, 1995). Hibiscus anthocyanins are phenolic natural pigments extracted from the dry calyces of *H. sabdariffa*. Anthocyanins, a subgroup of the flavonoids, are water soluble glycosides (Tsai *et al.*, 2002). Consumption of anthocyanins has been found to reduce the risk of coronary heart disease and prevent some chronic diseases (Renaud and De Logeril, 1992; Morazzoni and Bombardelli, 1996).

The increasing risk of exposure to cadmium makes the study of its toxicity not only important but also makes the study of ways of ameliorating its toxic effect imperative. While chelating therapy provides a means for treating

cadmium intoxication, it is relatively ineffective during long term exposures (Casarett and Doull, 1986). Since cadmium has been shown to exert its toxic effect via oxidative damage; this study was designed to evaluate the possible protective effects of *H. sabdariffa* aqueous extracts on some oxidative damage markers in rats exposed to cadmium.

## MATERIALS AND METHODS

This study was conducted from October to December, 2005 at the Department of Biochemistry, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria.

**Chemicals and reagents:** Cadmium Sulphate ( $\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ ) (E. Merck Darmstadt, Germany), adrenaline, trichloroacetic acid, thiobarbituric acid, bovine serum albumin (Sigma, London), other analytical grade chemicals were products of BDH Chemical Limited, Poole, England.

**Plant materials and preparation of extract:** *Hibiscus sabdariffa* plants were purchased from Oba Market, Ikare-Akoko, Ondo State. The plants were botanically authenticated by Dr. A.O. Obembe of the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba-Akoko, Ondo State.

The calyces were air dried and the *H. sabdariffa* extract was prepared by boiling 100 g of dried petal in 1 L of distilled water for 15 min, the extract was quantified and appropriate volumes were administered to the test animals.

**Experimental animals and management:** Twenty eight Male Wistar albino rats, weighing between 170-200 g, obtained from the Animal Unit of the Lagos University Teaching Hospital (LUTH), Lagos, Nigeria, were used for this study. The animals were fed with commercial feed (Product of Bendel Feed and Flour Mill, Ewu, Edo State) and water *ad libitum*. The research was conducted in accordance with the principles of laboratory animal use and care as found in the US guidelines (NIH publications No. 85-23, revised in 1985). The rats were divided into four groups: group I (Control), group II (*H. sabdariffa*), group III (Cadmium) and group IV (*H. sabdariffa*+Cadmium). Groups II and IV were given a daily dose of  $0.2 \text{ g kg}^{-1}$  body weight of *H. sabdariffa* extract for four weeks, while groups III and IV (the Cd groups) were injected sub-cutaneously with  $0.002 \text{ g Cd}$  (as  $\text{CdSO}_4 \cdot 8\text{H}_2\text{O}$ )  $\text{kg}^{-1}$  body weight once a week for four weeks. Animals were sacrificed 24 h after the last treatment. Blood was collected, allowed to clot and

serum was separated at 5000 rpm for 5 min and biochemical investigations were carried out. The liver, kidneys and testes were collected. The tissues were homogenized in ice cold physiological saline (1:4 w/v) and then centrifuged.

### Biochemical analysis

**Total protein:** Protein levels were determined in the serum, liver, kidney and testes by the Biuret method (Gornall *et al.*, 1949).

**Lipid peroxidation:** The malondialdehyde (MDA) level was used to estimate the level of lipid peroxidation. MDA levels were determined in the serum, liver, kidney and testes by the Thiobarbituric Acid Reactive Substances (TBARS) method (Varshney and Kale, 1990).

**Catalase:** Catalase activity was determined in the serum, liver, kidney and testes by the method of Sinha (1972).

**Superoxide dismutase (SOD):** Superoxide dismutase activity was determined in the serum, liver, kidney and testes by the method of Misra and Fridovich (1972).

**Statistical analysis:** The data are expressed as Mean $\pm$ SEM. The differences among means were analyzed by one-way ANOVA; inter-group comparisons were done using Duncan's Multiple Range Test (DMRT) with 95% confidence intervals. The SPSS 11.0, SPSS Inc., Chicago, Illinois, USA, was used for this analysis.

## RESULTS AND DISCUSSION

Cadmium is a potent cell poison known to cause oxidative stress by increasing lipid peroxidation and/or by changing intracellular glutathione levels (Figueiredo-Pereira *et al.*, 1998). The heavy metal is a substantial industrial and environmental pollutant that seriously injures a variety of organs, such as the brain, liver, testis and kidneys (Figueiredo-Pereira *et al.*, 1998).

**Body weight and relative organ weight:** Table 1 and 2 show the results for body weights and relative organ weights, respectively. The overall toxic effects of continuous exposure to Cd, *H. sabdariffa* and a combination of both, were assessed by monitoring rat

Table 1: Effect on body weight (g) of rats

Groups	Week 1	Week 2	Week 3	Week 4
I	183.88 $\pm$ 12.62	195.84 $\pm$ 15.37	201.78 $\pm$ 16.91	204.52 $\pm$ 16.39
II	171.50 $\pm$ 19.67	167.86 $\pm$ 21.46	175.12 $\pm$ 26.84	178.18 $\pm$ 24.26
III	173.14 $\pm$ 15.51	187.66 $\pm$ 17.21	174.90 $\pm$ 17.21	171.34 $\pm$ 16.97
IV	183.03 $\pm$ 24.07	188.15 $\pm$ 33.05	189.38 $\pm$ 28.00	187.78 $\pm$ 24.08

Values are expressed as Means $\pm$ SEM

**Table 2: Effect on relative organ weight of rats**

Groups	Relative liver weight	Relative kidney weight	Relative testis weight
I	0.0285±0.00154	0.00354±0.000188	0.00495±0.000381
II	0.0280±0.00183	0.00344±0.000357	0.00456±0.000100
III	0.0348±0.00157 <sup>a</sup>	0.00370±0.000128	0.00247±0.000260 <sup>a</sup>
IV	0.0340±0.00100 <sup>a</sup>	0.00365±0.000125	0.00257±0.000256 <sup>a</sup>

Values are expressed as Means±SEM. Values carrying any notation are significantly (p<0.05) different from control (group I), those carrying different notations are statistically (p<0.05) different from other test groups

**Table 3: Effect on protein levels (mg mL<sup>-1</sup>) of rats**

Groups	Serum	Liver	Kidney	Testis
I	1.150±0.0865	0.788±0.322	0.124±0.0192	0.290±0.177
II	1.580±0.348 <sup>a</sup>	0.510±0.062 <sup>a</sup>	0.370±0.129 <sup>a</sup>	0.333±0.034 <sup>a</sup>
III	1.090±0.193	0.526±0.145 <sup>a</sup>	0.223±0.056 <sup>a</sup>	0.317±0.140 <sup>a</sup>
IV	0.352±0.143 <sup>b</sup>	0.566±0.076 <sup>b</sup>	0.263±0.072 <sup>a</sup>	0.397±0.051 <sup>b</sup>

Values are expressed as Means±SEM. Values carrying any notation are significantly (p<0.05) different from control (group I), those carrying different notations are statistically (p<0.05) different from other test groups

body weight gain and organ-body weight ratio. The reduction in the body weights of all test animals compared to control indicates that *H. sabdariffa*, cadmium and cadmium with *H. sabdariffa*, in the doses given, had weight reducing effects on test animals. Cadmium has been shown to cause reductions in body weight (Ribas *et al.*, 2004; Asagba *et al.*, 2006; Grosicki and Kowalski, 2002). Reductions in the body weight of rats given *H. sabdariffa* have also been reported by Asagba *et al.* (2006). Cadmium, as Cd alone or in combination with *H. sabdariffa* caused an enlargement of the liver, as recorded by the significantly (p<0.05) increased relative liver weights of groups III and IV. Staneviciene *et al.* (2006) reported an increase in liver body weight ratio of mice exposed to Cd, ultra-structural alterations, such as a thicker capsule and heaps of immune cells in liver were observed in their study. The increase in relative liver weight is not surprising since the liver accumulates substantial amounts of administered Cd (Grosicki and Kowalski, 2002). Cd, *H. sabdariffa* and the combination of both, had no effect on the relative weight of the kidneys. The reduction in the relative testis weight of the Cd exposed groups is a reflection of the established testis toxicity of Cd. *H. sabdariffa* alone had no effect on the liver, kidney and testes weights.

**Protein estimation:** Table 3 shows protein estimation for serum, liver, kidney and testes. The most obvious reduction in total protein levels was seen in liver protein, where, Cd, *H. sabdariffa* and the combination of both, showed significant (p<0.05) decreases. This could be a reflection of the toxicity of accumulated Cd and *H. sabdariffa* in the liver, which receives and processes most ingested materials. The cadmium-induced thiolation of protein-SH groups leading to the formation of protein-mixed disulfide, may provoke the misfolding of

**Table 4: Effect on malondialdehyde levels (units mL<sup>-1</sup>×10<sup>-6</sup>) of rats**

Groups	Serum	Liver	Kidney	Testis
I	11.48±3.05	9.15±2.05	2.62±0.48	2.78±1.06
II	19.65±4.35 <sup>b</sup>	11.36±2.16 <sup>a</sup>	4.96±1.20 <sup>a</sup>	8.23±1.11 <sup>c</sup>
III	13.49±2.35 <sup>a</sup>	23.05±0.53 <sup>b</sup>	2.61±0.37	10.62±1.98 <sup>b</sup>
IV	11.25±3.71	9.68±2.91	5.87±2.12 <sup>b</sup>	5.29±1.80 <sup>a</sup>

Values are expressed as Mean±SEM. Values carrying any notation are significantly (p<0.05) different from control (group I), those carrying different notations are statistically (p<0.05) different from other test groups

proteins, which may then be targeted for degradation by the ubiquitin/ATP-dependent proteolytic pathway (Figueiredo-Pereira *et al.*, 1998). The significant (p<0.05) increases observed in kidney and testes protein levels could be due to the increase in the production of enzymes, especially anti-oxidant enzymes (Gupta *et al.*, 1991), in this study, significant (p<0.05) increases in kidney and testis SOD activities were observed.

**MDA levels:** Table 4 shows the level of lipid peroxidation, measured as MDA levels, in the serum, liver, kidney and testes. Cd has been shown to increase lipid peroxidation in the brain, an organ particularly sensitive to cadmium toxicity (Acan and Tezcan, 1995) and in hepatocytes and testicular Leydig cells (Muller, 1986; Koizumi and Li, 1992), this correlates well with the general increases observed in both the tissue and serum malondialdehyde levels of test animals in this study. Group III, given Cd only, recorded the highest MDA levels in the liver and the testes; this again, is not surprising since these tissues are particularly susceptible to Cd toxicity. In these tissue also, as well as in the serum, the *H. sabdariffa* supplemented Cd group had significantly (p<0.05) lower MDA levels than the Cd treated group, indicating that *H. sabdariffa* could be protective against Cd-induced lipid peroxidation. *In vivo* investigation in rats, showed that oral pre-treatment with *H. sabdariffa* for five days significantly lowered the serum levels of hepatic enzymes markers and reduced oxidative liver damage (Wang *et al.*, 2000). Surprisingly, however, the group given only *H. sabdariffa* had the highest MDA level in the serum, as well as significantly (p<0.05) higher levels relative to control in the liver, kidney and testis, implying that *H. sabdariffa* alone, in the dose used in this study may exert some measure of oxidative damage. Akindahunsi and Olaleye (2003) reported that administration of high doses of *H. sabdariffa* could cause liver damage.

**Catalase activity:** The kidney enzyme showed the most obvious decrease in catalase activity (Table 5). In animal tissues, it has been demonstrated that cadmium induces changes in the antioxidant status either by increasing superoxideradical production and lipid peroxidation, or by decreasing the enzymatic and non-enzymatic antioxidants (Stohs and Bagchi, 1995). The decreases observed in catalase activity in this study could therefore be due to

Table 5: Effect on catalase activity ( $\mu\text{mol}$  of  $\text{H}_2\text{O}_2$  decomposed/min/mg protein) of rats

Groups	Serum	Liver	Kidney	Testis
I	7.895 $\pm$ 1.676	4.732 $\pm$ 3.358	345.450 $\pm$ 55.564	26.346 $\pm$ 8.901
II	2.936 $\pm$ 0.891 <sup>a</sup>	2.568 $\pm$ 0.746 <sup>a</sup>	42.258 $\pm$ 14.851 <sup>a</sup>	16.206 $\pm$ 6.252 <sup>b</sup>
III	7.702 $\pm$ 3.437	4.767 $\pm$ 1.426 <sup>b</sup>	62.597 $\pm$ 20.842 <sup>a</sup>	27.360 $\pm$ 6.070
IV	24.528 $\pm$ 7.898 <sup>b</sup>	1.603 $\pm$ 0.223 <sup>b</sup>	49.836 $\pm$ 14.771 <sup>a</sup>	20.426 $\pm$ 5.185 <sup>a</sup>

Values are expressed as Means $\pm$ SEM. Values carrying any notation are significantly ( $p < 0.05$ ) different from control (group I), those carrying different notations are statistically ( $p < 0.05$ ) different from other test groups

Table 6: Effect on SOD activity (units/mL/mg protein) of rats

Groups	Serum	Liver	Kidney	Testis
I	1607.14 $\pm$ 10.31	571.45 $\pm$ 14.29	725.68 $\pm$ 23.50	357.14 $\pm$ 10.00
II	982.14 $\pm$ 30.50 <sup>b</sup>	1071.31 $\pm$ 20.62 <sup>a</sup>	952.38 $\pm$ 17.66 <sup>b</sup>	476.19 $\pm$ 15.29 <sup>a</sup>
III	918.37 $\pm$ 20.41 <sup>c</sup>	765.31 $\pm$ 14.43 <sup>b</sup>	969.39 $\pm$ 20.19 <sup>b</sup>	918.37 $\pm$ 13.73 <sup>c</sup>
IV	1214.29 $\pm$ 18.21 <sup>a</sup>	773.81 $\pm$ 17.05 <sup>a</sup>	833.33 $\pm$ 21.95 <sup>a</sup>	536.71 $\pm$ 12.20 <sup>b</sup>

Values are expressed as Means $\pm$ SEM. Values carrying any notation are significantly ( $p < 0.05$ ) different from control (group I), those carrying different notations are statistically ( $p < 0.05$ ) different from other test group

the Cd-induced oxidative damage. The fact that *H. sabdariffa* alone also caused a decrease in tissue and serum catalase implies that it affects oxidant-antioxidant equilibrium of the experimental animals.

**SOD activity:** The serum SOD levels for all tests groups were significantly ( $p < 0.05$ ) lower than control. The liver SOD activity of all tests groups were significantly ( $p < 0.05$ ) higher than control. This same trend is seen in kidney and testes SOD activity (Table 6). The significant ( $p < 0.05$ ) increases observed in all the test groups for liver, kidney and testis superoxide dismutase activities is a reflection of the anti-oxidant response to oxidative damage. Cells usually increase the production of anti-oxidant enzymes such as glutathione peroxidase and superoxide dismutase, in order to counter the effects of oxidative stress (Gupta *et al.*, 1991). The significant ( $p < 0.05$ ) decreases observed in all the test groups in serum SOD activity, especially the Cd treated group, which showed the lowest decrease, could be due to the early inhibitory effect of Cd on SOD activity (Bagchi *et al.*, 1996; Gupta *et al.*, 1991; Sarkar *et al.*, 1995).

## CONCLUSION

The findings from this study have shown that sub-cutaneous injection of Cd and continuous oral administration of *H. sabdariffa*, caused reduction in body weights of rats, Cd alone or in combination with *H. sabdariffa* increased relative liver weight, decreased relative testes weight, decreased liver protein levels, while testes and kidney protein levels were increased. Increase in serum and tissue MDA levels, increase in liver, kidney and testis SOD activities and decrease in kidney catalase activities were also observed in all test groups. For some parameters evaluated, especially estimation of lipid peroxidation, the results showed that *H. sabdariffa* played

a protective role against Cd-induced oxidative damage. Again, the results indicate, that under the conditions of this study, the liver and testis were largely affected by Cd-induced oxidative damage and the protective effect of *H. sabdariffa* were also more pronounced in these tissues. Paradoxically, *H. sabdariffa* alone appeared to exert some measure of oxidative damage under the conditions of this study, implying that continuous high doses of *H. sabdariffa* could have a negative effect.

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