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Protective role of whole and anthocyanin-free aqueous extracts of *Hibiscus sabdariffa* L. on cadmium-induced prostate, testicular and nephro toxicity markers in male wistar rats

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ABSTRACT

Cadmium is one of the extremely toxic metals commonly found in industrial work places with very low permissible exposure limit (PEL). Exposure to cadmium has been associated with organ toxicity which can culminate in cancer of the prostate and testis. Cadmium elicits its toxicity via free radical generation and associated oxidative damage. Some plant extracts are known to contain anti-oxidant pigments. The influence of whole and anthocyanin free aqueous extracts of *Hibiscus sabdariffa , L.* on cadmium- induced prostate, testicular and nephro toxicity in male wistar rats was studied using a combination of organ: body weight ratio, total and prostatic acid phosphatase activity, organ superoxide dismutase activity, catalase activity, and malondialdehyde (MDA) levels. Cadmium treated rats had significantly ($P < 0.05$)increased malondialdehyde (MDA) levels but decreased prostate / testis:body weight ratio, when compared with the control values. The groups treated with *Hibiscus Sabdariffa, L* whole aqueous extract prior to cadmium exposure had low malondialdehyde levels but increased prostate/ testis:body weight ratio when compared with rats in cadmium treated group and control. The group of rats that received the *Hibiscus sabdariffa* anthocyanin –free extract also had low malondialdehyde levels but increased organ body weight ratio when compared with rats in the cadmium only treated and control groups. Prior administration of *Hibiscus sabdariffa L*. whole and anthocyanin free extracts reduced testicular toxicity caused by cadmium exposure

Key words: Hibiscus sabdariffa, L., Cadmium, Acid phosphatase, Catalase, Superoxide dismutase (SOD), Malondialdehyde (MDA).

INTRODUCTION

Due to the high oil exploration and exploitation activities in the Niger- Delta region of the South-South, Nigeria, the environment, which includes flora and fauna, plants, animals, air, water and soil are almost continuously being exposed to noxious substances of proven toxicological effect.

Of special concern among these noxious environmental pollutants are the heavy metals like cadmium and lead (Gonzalez – Villalva, *et al*., 2006). Cadmium has been reported to be a hazardous metal commonly found in industrial work places. It is particularly associated with exploration and exploitation activities like welding and mining. Cadmium is of special concern because of its low permissible exposure limit (PEL), that is, at very trace and insignificant quantities, there have been reports of toxicity which include : gonadotoxic and spermatotoxic effects, cancer of prostate and testis, hypogonadism and infertility, gastroenteritis,

hypertension(Lall, *et al.,* 1997), hepatotoxicity, nephrotoxicity (Horiguchi, *et al.,* 1996). Bone disorders have been observed in some cases of cadmium toxicity (Ryan, *et al*., 2000).

In man, the toxic effect of cadmium are by mechanism connected to its ability to generate free radicals at a rate high enough to overwhelm the natural antioxidant defence systems of the body (Bagchi, *et al*., 1996; Adaikpoh, *et al*., 2007) and its ability to inactivate enzymes containing sulfydryl groups (Timbrell, 1991) and uncouple oxidative phosphorylation in mitochondria (Telisma, *et al*., 2000).

Although, the use of nose mask and respirators by oil and gas workers have been adopted as a way of mitigating the effects of direct inhalation of dust particles contaminated with heavy metals including cadmium (since one of its portal route to the body is via inhalation),successes achieved so far, have not been encouraging.

This study investigates the use of nature's own cheap and easily available antioxidant -rich drink made from *Hibiscus sabdariffa* as an *in vivo* protective (prophylactic) substance, which could be used to augment the safety devices already adopted in industries to reduce exposure to heavy metal contamination.

MATERIAL AND METHODS

Animals

Forty- eight (48) adult male albino rats (wistar strain) with an initial weight of 110±28g (90-140g) were used for the study.

The rats were bred in the School of Veterinary Medicine Department, University of Nigeria, Nsukka, Enugu State, Nigeria. The rats were housed in steel cages under standard conditions in the Biochemistry Department of the University of Benin, Nigeria. They were maintained on growers' mash and water *ad libitum* and left to acclimatize to the laboratory condition for five (5) weeks before the commencement of the study.

Plants

Dried *calyces of Hibiscus sabdariffa,* L. locally known as 'zobo' were sourced from Hausa Quarters, Warri, Nigeria.

Preparation of whole aqueous Extract of *H. sabdariffa* L

Dried calyces of *Hibiscus sabdariffa* (100g) were soaked in 600ml of cold deionized water for 4 hrs, filtered and a red pigment was obtained and the residue discarded. The filtrate was made up to 1000ml with deionized water. The solid residue of the filtrate was determined by drying 1ml of the filtrate at 200°C in a preweighed watch glass. The solid content was found to be 51.6mg/ml. A 5% (v/v) solution of the extract was then made.

Preparation of anthocyanin free extract

The anthocyanin-free extract of *Hibiscus sabdariffa* was prepared by passing the whole extract of *Hibiscus sabdariffa* through a cation exchanger in a short chromatographic column.

Experimental groups

The rats were given the whole extract and anthocyanin-free extract of *Hibiscus sabdariffa L*. prior to the induction of testicular toxicity by daily intraperitoneal injection of cadmium at a dose of 100mg/kg body weight for 2 weeks.

The rats were divided into 6 groups of 8 rats each. Group 1 (control) rats were given deionized water only (5ml/kg body weight). Group 2 rats were given whole aqueous extract of the calyces (WE: 250mg/kg body weight) by gavage. Group 3 rats were rats orally given (WE: 250mg/kg body weight and cadmium, Cd: 3mgCd /kg body weight) intraperitoneally. Group 4 rats were treated with Cd only (3mg/kg body weight).Group 5 rats were given the anthocyanin-free whole *Hibiscus sabdariffa L.* extract (AFE: 100mg/kg body weight) by gavage and Group 6 rats were given (AFE: 100mg/kg body weight and Cd: 3mg/kg body weight) intraperitoneally.

The extracts were given to the appropriate rats once a day for two weeks while the Cd was administered once a week for two weeks.

At the end of the second week, the animals were anesthetized in a chloroform saturated chamber. The thoracic and abdominal regions were

opened to expose the heart and other organs. Blood was collected by directed cardiac puncture and transferred into heparinized tubes and centrifuged immediately at 3000rpm for 10mins at about 29°C, to obtain plasma. The plasma was stored frozen until required for assays. The kidneys, testes and prostate were excised, weighed and stored frozen until required.

Assays

Lipid peroxidation, as measured by malondialdehyde (MDA) content, was assayed by the TBA method of Beuge and Aust (1978).

Superoxide dismutase (SOD: EC 1.15.1.1) activity was measured by the method of Misra and Fridovich (1972).

Catalase (CAT: EC 1.11.1.6) activity was estimated by the method of Sinha (1971) by measuring the rate of decomposition of hydrogen peroxide (H_2O_2).

Acid phosphatase activity was determined in the prostate and plasma according to the method of Fishman, et al. (1953).

RESULTS

Results from the investigation are shown on Tables 1- 7. Table 1 shows changes in weight gain for the different experimental groups.

The body weight gain of rats given the whole extract plus Cadmium (WE +Cd), Cd only (Cd), and Anthocyanin free extract plus Cd (AfE + Cd) decreased significantly (P<0.05) relative to the control (deionized water) group.

Cadmium only (Cd) significantly (P<0.05) decreased the prostate: body weight ratio; testis: body weight ratio; and the kidney:body weight ratio of male rats in the respective test groups relative to the control but administration of the extracts, before cadmium exposure caused a significant (P<0.05) increase in kidney and prostate body weight ratio relative to the Cadmium only group. Though there was an increase in the testis body weight ratio, of rats given the two extracts prior to Cd administration, it was not statistically significant (P>0.05)

The influence of the extracts on plasma acid phosphatase of male rats exposed to cadmium is shown in (Table 3). A significant(P<0.05) decrease in the plasma prostatic acid phosphatase of male rats given Cadmium only (+Cd) was observed relative to the other test groups. Prior administration of the whole extract (AfE) before cadmium exposure significantly increased the prostate prostatic acid phosphatase activity relative to the Whole *Hibiscus sabdariffa* group (WE) and Cadmium only group.

The effects of the two different extracts on prostate acid phosphatase activity in rats exposed to cadmium are shown (Table 4).

The influence of the extracts on prostate acid phosphatase activity is presented in Table 4. A significant (P<0.05) increase is observed in the prostate prostatic acid phosphatase activity of rats given the whole *Hibiscus sabdariffa* extract relative

Table 1: Effects of Whole and Anthocyanin-Free Extracts on Body Weight Gain of rats exposed to cadmium

Group	Treatment	Body Weight Gain (g)Mean ± SEM (n=8)
1	Control	17.50 ± 1.20[a]
2	WE	13.20 ± 3.75[b]
3	WE + Cd	8.30 ± 2.16[b]
4	Cd	10.22 ± 7.29[b]
5	AFE	15.00 ± 2.59[b]
6	AFE + Cd	7.70 ± 2.59[b]

Control: 5ml H2O/kg body weight

WE: *Hibiscus sabdariffa,L*. whole aqueous extract (250mg extract/kg body weight)

WE + Cd: *Hibiscus sabdariffa,L*. whole aqueous extract (250mg extract/kg body weight) +Cadmium(3mg/kg body weight)

Cd: Cadmium3mg/kg body weight

AFE: Anthocyanin free *Hibiscus sabdariffa,L*. whole aqueous extract (100mg extract/kg body weight)

AFE + Cd: Anthocyanin free *Hibiscus sabdariffa,L*. whole aqueous extract (100mg extract/kg body weight) +Cadmium(3mg/kg body weight)

Values on the same row with different superscript from Cd only group differ significantly (P <0.05). values are expressed as mean ± SEM for n=8 per group.

to the control (deionized water) group and the cadmium only group. Prior administration of the extract before toxicity by cadmium increased the prostate prostatic acid phosphatase activities of the group given whole *Hibiscus sabdariffa* extract relative to the cadmium only group. There was also a significant increase in the prostate prostatic acid phosphatase activity in the groups administered with

Table 2: Effect of whole and anthocyanin free extracts on Organ-body weight ratio of rats exposed to cadmium

Group.	Treatment	Organ: body weight Ratio(Mean ±SEM)×10^{-3} (n=8)		
		Kidney	Prostate	Testis
1	Control	9.50 ± 0.39[a]	6.40±0.08[a]	2.46±0.06[a]
2	WE	8.60±0.42[a]	5.50±0.02[a]	2.00 ±0.12[a]
3	WE +Cd	8.40±0.47[a]	5.20±0.06[a]	1.91±0.11[b]
4	Cd)	6.70 ± 0.57[b]	2.57 ± 0.09[b]	1.13 ± 0.06[b]
5	AfE	8.50± 0.26[a]	4.40±0.08[b]	1.77±0.09[b]
6.	AfE +Cd	7.50 ± 0.11[b]	3.90 ±0.002[b]	–1.50±0.08[b]

Values on the same row with different superscript from Cd only group differ significantly (P <0.05).
a= values are significantly different from Cd only group.
b= values are not significantly different from Cd only group
Control: 5ml H2O/kg body weight
WE: *Hibiscus sabdariffa,L*. whole aqueous extract (250mg extract/kg body weight)
WE + Cd: *Hibiscus sabdariffa,L*. whole aqueous extract (250mg extract/kg body weight) +Cadmium(3mg/kg body weight)
Cd: Cadmium3mg/kg body weight
AFE: Anthocyanin free *Hibiscus sabdariffa,L*. whole aqueous extract (100mg extract/kg body weight)
AFE + Cd: Anthocyanin free *Hibiscus sabdariffa,L*. whole aqueous extract (100mg extract/kg body weight) +Cadmium(3mg/kg body weight)

Table 3: Changes in plasma acid phosphatase activity in rats receiving cadmium and *H.Sabdariffa* extracts

Group	Treatment	Enzyme Activity (U/ml)	
		Total Acid Phosphatase	Plasma Prostatic Acid Phosphatase
1.	Deionized H_2O (5ml/kg bd wt)	36.50±5.60[a]	8.80±1.20[a]
2.	Whole extract (WE) (250mgWE/kg bd wt)	10.20±1.30[b]	4.70±0.60[b]
3.	WE +Cadmium (Cd) 250mg WE/kg bd wt + 3 mg Cd/kg bd wt)	6.50±0.80[b]	3.00±0.50[b]
4.	Cd (3mg Cd/kg bd wt)	7.30±0.50[b]	3.30±0.50[b]
5.	Anthocyanin free Extract(AfE) (100mg AfE/kg bd wt)	17.50±5.40[b]	6.30±3.00[b]
6.	AfE +Cd (100mg AfE/kg bd wt +3mg Cd/kg bd wt)	46.90±3.10[a]	17.4±3.9[a]

Values on the same row with different superscript from Cd only group differ significantly (P < 0.05).
a= values are significantly different from Cd only group.
b= values are not significantly different from Cd only group
values are expressed as Mean± SEM for n=8 rats per group.

anthocyanin free extracts relative to the cadmium only group.

The effects of the two different extracts on superoxide dismutase activity, catalase activity and malondialdahyde level in the kidney, prostate and testes of cadmium exposed rats are presented in Tables 5 -7, respectively.

The effects of the extracts on rat organ superoxide dismutase activity are presented in Table 5. A significant (P<0.05) increase in SOD activity was observed in the organs (kidney, prostate and testis) of the group administered cadmium only relative to the other groups. The groups given the *Hibiscus sabdariffa* whole extract and the anthocyanin -free extract showed significant increase in their superoxide dismutase activity relative to the control (deionized water) group.

Table 4: Effects of whole and anthocyanin-free extracts on prostate acid phosphatase activity in rats exposed to cadmium

Group	Treatment	Enzyme Activity (U/ml)	
		Total Acid Phosphatase	Plasma Prostatic Acid Phosphatase
1.	Deionized H_2O (5ml/kg bd wt)	3.68±0.28[b]	1.53±0.06[a]
2.	Whole extract (WE) (250mgWE/kg bd wt)	5.49±0.54[a]	2.32±0.21[a]
3.	WE +Cadmium (Cd) 250mg WE/kg bd wt + 3 mg Cd/kg bd wt)	5.46±0.45[a]	1.31±0.48[b]
4.	+Cd (3mg Cd/kg bd wt)	3.16±0.36[b]	0.60±0.08[b]
5.	Anthocyanin free Extract(AfE) (100mg AfE/kg bd wt)	6.54±1.02[a]	1.78±0.08[a]
6.	AfE +Cd (100mg AfE/kg bd wt +3mg Cd/kg bd wt)	3.21±0.69[b]	0.93±0.31[b]

Values on the same row with different superscript from Cd only group differ significantly (P <0.05).

a= values are significantly different from Cd only group.

b= values are not significantly different from Cd only group

values are expressed as Mean± SEM for n=8 rats per group.

Table 5: Effects of whole and anthocyanin-free extracts on superoxide dismutase activity in rats exposed to cadmium

Group	Treatment	SOD Activity (units/g tissue)		
		Kidney	Prostate	Testis
1	+ Deionized H_2O(5ml/kg bd wt)	2.01±0.23[a]	5.73±0.75[b]	1.45±0.05[a]
2	Whole extract (WE)(250mgWE/kg bd wt)	2.10±0.16[a]	6.90±0.87[b]	1.73±0.24[a]
3	WE +Cadmium (Cd) 250mg WE/kg bd wt + 3 mg Cd/kg bd wt)	4.38±1.75[b]	5.13±0.45[a]	2.53±0.04[a]
4	+Cd (3mg Cd/kg bd wt)	5.67±1.18[b]	13.12±1.93[b]	5.28±0.07[b]
5	Anthocyanin free Extract(AfE) (100mg AfE/kg bd wt)	4.65±0.89[b]	2.60±0.16[a]	2.73±0.72[b]
6.	AfE +Cd(100mg AfE/kg bd wt +3mg Cd/kg bd wt)	7.50±1.80[b]	2.39±0.09[a]	4.75±0.53[b]

Values on the same row with different superscript from Cd only group differ significantly (P <0.05).

a= values are significantly different from Cd only group.

b= values are not significantly different from Cd only group

values are expressed as Mean± SEM for n=8 rats per group.

The influence of the extracts on tissue malondialdehyde levels is presented in Table 6.There was a significant(P< 0.05) decrease in the malondialdehyde levels of groups given the whole *Hibiscus sabdariffa* only and the anthocyanin free whole *Hibiscus sabdariffa* relative to the control. Prior administration of the extracts before cadmium poisoning significantly decrease the Malondialdehyde (MDA) levels relative to the cadmium only group.

The effects of the extracts on tissue catalase activity are presented in Table 7.The catalase activity of the group administered *Hibiscus sabdariffa* whole extract was observed to increase significantly (P<0.05) relative to the control (deionized water) group, in the kidney, prostate and testis of the male rats. There was a slight increase in catalase activity of the groups given the extracts prior to cadmium exposure when compared with cadmium group.

Table 6: Effects of whole and anthocyanin-free extracts on TBARS levels in rats exposed to cadmium

Group	Treatment	Malondialdehyde levels (µmole MDA/g tissue) (Mean ±SEM)×10^{-3} (n=8)		
		Kidney	Prostate	Testis
1	+ Deionized H_2O(5ml/kg bd wt)	2.60±0.06[a]	1.73±0.18[a]	1.58±0.09[b]
2	Whole extract (WE)(250mgWE/kg bd wt)	2.22±0.14[a]	1.43±0.12[a]	1.30±0.05[a]
3	WE +Cadmium (Cd) 250mg WE/kg bd wt + 3 mg Cd/kg bd wt)	3.08±0.12[b]	3.95±0.21[b]	6.53±0.17[b]
4	+Cd (3mg Cd/kg bd wt)	5.52±0.11[b]	8.40±0.16[b]	18.30±0.71[b]
5	Anthocyanin free Extract(AfE) (100mg AfE/kg bd wt)	2.19±0.24[a]	1.74±0.14[a]	1.37±0.07[a]
6.	AfE +Cd(100mg AfE/kg bd wt +3mg Cd/kg bd wt)	3.20±0.25[b]	4.98 ± 0.18[b]	6.51 ± 0.18[b]

Values on the same row with different superscript from Cd only group differ significantly (P <0.05).

a= values are significantly different from Cd only group.

b= values are not significantly different from Cd only group

Table 7: Effects of whole and anthocyanin-free extracts on Catalase Activity in rats exposed to cadmium

Group	Treatment	Catalase Activity		
		Kidney	Prostate	Testis
1	Deionized H_2O(5ml/kg bd wt)	15.59±0.22[a]	3.83±0.07[a]	31.12±0.45[a]
2	Whole extract (WE)(250mgWE/kg bd wt)	17.86±0.24[a]	4.04±0.06[a]	35.64±0.47[a]
3	WE +Cadmium (Cd) 250mg WE/kg bd wt + 3 mg Cd/kg bd wt)	9.41±0.09[b]	2.62±0.16[b]	24.50±0.73[b]
4	Cd (3mg Cd/kg bd wt)	5.91±0.18[b]	1.84±0.11[b]	14.85±0.34[b]
5	Anthocyanin free Extract(AfE) (100mg AfE/kg bd wt)	10.30±0.34[b]	3.31±0.1[b]	27.27±0.77[b]
6.	AfE +Cd(100mg AfE/kg bd wt +3mg Cd/kg bd wt)	7.19±0.19[b]	2.89±0.06[b]	18.00±0.76[b]

Values on the same row with different superscript from Cd only group differ significantly (P <0.05).

a= values are significantly(P<0.05) different from Cd only group.

b= values are not significantly (P>0.5) different from Cd only group

values are expressed as Mean± SEM for n=8 rats per group.

DISCUSSION

Alteration in body weight gain (Table1) and organ: body weight ratio (Table 2) is usually used as indices of toxicity, (Timbrell, 1991). The significant decrease in body weight of rats given cadmium only, could be due to cadmium toxicity. These findings agree with previous reports (Asagba, *et al.,* 2007; Horiguchi, *et al*., 1996) .There was no significant decrease in weight gain of rats given the whole extract only when compared with the control. The anthocyanin free extract did not alter the body weight gain of the rats significantly .Also prior administration of either the whole or anthocyanin free extract to the rats before exposure to Cd did not prevent the cadmium induced significant loss in body weight (Table 1),and this is in accordance with an earlier report (Asagba,*et al*., 2007).

Thus, the ability of the whole extract to ameliorate cadmium toxicity may not be due to the presence or absence of anthocyanin.

Examination of Table 2, reveals that rats exposed to cadmium alone had significantly ($P<0.05$) reduced kidney, prostate and testes body weight ratios, when compared with corresponding organ-body weight ratios obtained from the control rats. This effect was reversed when rats were exposed to whole aqueous extract (WE) prior to Cd intoxication, but the anthocyanin-free extract (AfE) did not prevent Cd-induced reduction in the organ-body weight ratios. It does appear therefore that anthocyanin is an essential component of the whole aqueous extract of *H. Sabdariffa L* necessary for the impairment of this particular effect of Cd in the selected rat organs examined in this study.

Cadmium impaired acid phosphatase activity. This is evident from its action on plasma total and plasma prostatic acid phosphatase activities (Table 3); This, however, is not in consonance with earlier report (Asagba, *et al*., 2007), in which the workers found no difference in the activity of plasma prostatic acid phosphatase when the mean activity values of the Cd – exposed rats were compared with those of water treated controls. In this study, as in that of Asagba, *et al,* (2007), WE of *H. sabdariffa* L. petals did not alter the activity of prostatic acid phosphatase when administered to the rats prior to Cd intoxication, but unlike WE, AfE when given to rats before Cd-exposure caused statistically significant ($P < 0.05$) increased in both plasma total and plasma prostatic acid phosphatase activities, though to levels that were not significantly ($P > 0.05$) different from that of water-treated control. Evidently, the ability of Cd to inhibit those enzymes was prevented by the AfE. This implies that anthocyanin contributes markedly well to what mechanism Cd uses in the inhibition of the isoforms of plasma acid phosphatase.

Prostate prostatic acid phosphatase activity was inhibited by Cd alone (Table 4). Prior exposure of separate rats to WE and AfE respectively prevented this action of Cd.

Again, AfE had profound effect on the ability of CD to inhibit prostatic acid phosphatase.

In the kidney, prostate and testes, Cd significantly increased ($P < 0.05$) SOD activity when compared with water treated control (Table 5). Unlike its effect on SOD activity such Cd significantly ($P < 0.05$) decreased the activity of catalase in the kidney, prostatic and testes of Cd-only treated rats (Table 6). In response to oxidative stress cells increase their production of antioxidant enzymes such as catalase, glutathione peroxidase and SOD (Gapta, *et al*, 1991). However, it has been abundantly demonstrated that the early effects of Cd exposure is the inhibition of SOD (Bagahi ,*et al*., 1996; Gupta, 1991; Jakar, *et al*., 1995).

In this study, Cd did not inhibit SOD activity, but rather it inhibited catalase activity. It is interesting though to observe that WE and AfE counteracted Cd-induced increase in SOD activity in the prostate and testes. These extracts (WE and AfE) also counteracted the Cd – induced reduction in kidney, prostate and testis catalase activity (Tables 5 and 6). This observation is in agreement with the fact that kidney, prostate and testes from Cd-only treated rats had elevated lipid peroxidation (Table 7), whereas in rats pre-treated with WE and AfE before Cd administration, there was reduced SOD activity, increased catalase activity and corresponding low MDA level, a lipoperoxidation index (Table 5, 6 and 7). These observations

indicate that the extract from *H. sabdariff* L. possesses bioactive agents that protected these organs from the effect of cadmium.

The WE of *H. Sabdariffa, L.* contains anthocyanin, a flavonoids, vitamin C and E (Christie, 1984). These are established antioxidants. Flavonoids decrease Cd concentration in tissues (Kowalczy, *et al*., 2003). It is therefore likely that this is one mechanism by which the whole extract counters the biochemical effect of Cd.

Overall, this study indicates that the extracts from *H. sabdariff L*. calyces offered protection against cadmium–induced toxicity in some organs and tissues of male rats.

Thus, the intake of whole extract popularly known as "Zobo" could confer additional measure of protection to cadmium- induced toxicity especially among workers exposed to the risk of such toxicity. Nevertheless, the active ingredients should be isolated, purified and characterized for possible therapeutic applications.

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