Response of Oxidative Enzymes to *Hibiscus sabdarriffa* L. anthocyanins Treatment in Cadmium-exposed Rats

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Abstract. *Background:* Animal studies on the effect of cadmium on aldehyde oxidase (AO), xanthine oxidase (XO), and sulphite oxidase (SO) are few and despite the huge potentials of anthocyanins in ameliorating Cd toxicity, reports on the response of these enzymes in Cd-exposed rats treated with anthocyanins are scarce. Therefore, this was designed to investigate the response of oxidative enzymes to *Hibiscus sabdarriffa* L. anthocyanins (HSA) treatment in cadmium-exposed rats. Anthocyanins were extracted from *H. sabdariffa* calyces using standard methods. One hundred and twenty six adult male wistar rats were used for the study. They were divided into eighteen (18) groups with seven rats in each group. The experimental groupings were: A: Control, B: Cd, C: HS aqueous extract, D: HSA, E: HSA Pre-Cd and F: HSA Post-Cd, with groups D-F having sub-groups of high and low doses. The rats were treated for two experimental periods: 5 days (acute) and 15 days (sub-chronic), after which they were sacrificed for biochemical analysis.

Results: Exposure to Cd caused significant reduction in the activities of AO, XO and SO in the plasma, epididymis, liver, kidney, prostrate and testis. However, pre-treatment and post-treatment of Cd-exposed rats with *H. sabdariffa* anthocyanins significantly ($p>0.05$) reversed the toxic effects of Cd-exposure in most of the tissues and parameters examined. This effect was seen to be dose and duration of exposure dependent as well as both curative and protective. However pretreatment and high doses of HSA resulted in higher induction of the activity of oxidative enzymes.

Conclusions: The findings indicate that the administation of *H. sabdariffa* anthocyanins can induce the activities of oxidative enzymes, which are required to counter the toxic effects of xenobiotics and heavy metals such as Cd.

Keywords: xanthine oxidase, aldehyde oxidase, sulphite oxidase, anthocyanins

Background

Oxidative enzymes such as aldehyde, xanthine, monoamine and sulphite oxidases, play huge roles in protecting cells and tissues against the harmful effects of xenobiotics, chemicals and toxicants by converting them into less harmful compounds that can easily be excreted (Roesijadi, 1996; Tutuncu *et al.*, 2012; Montefiori *et al.*, 2017; Ekakitie *et al.*, 2019). This process, known as, biotransformation takes place mainly in the liver but also occurs in other tissues and organs and thus, these enzymes which contain molybdenum and haem are abundant in the liver (Timbrell, 2000; Beedham, 2002; Hille, 2005). The activities of these important enzymes have been shown to be negatively affected by heavy metals, one of which is cadmium, regarded as an environmental containment and carcinogen (Asagba, 2010; Ezedom & Asagba, 2016).

Cadmium (Cd) a transition metal, is ubiquitous in nature due to its numerous industrial applications, but is non-essential and toxic in plants, animals and humans (Tolcin, 2016; Das *et al.*, 2019). Cd has a long half life of 25–30 years, can easily be transferred from the soil to plants and thus accumulates in plants and animals leading to several toxic effects in kidneys, liver testes,, lung, bones, pancreas and other tissues and organs (Lamb *et al.*, 2016; Dhouha *et*

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al., 2017; Ismael *et al.,* 2019; Xuebin *et al.,* 2020; Genchi *et al.,* 2020). Cd toxicity is often manifested via induction of oxidative stress in cells and tissues (Cuypers *et al.,* 2010; Orororo *et al.,* 2018b).

To counter the effects of Cd, researchers have advocated the use of biologically active compounds that occur naturally in plants such as anthocyanins and other polyphenolic substances (Ibrahim *et al*., 2014; El-Boshy *et al.,* 2015; Orororo *et al.,* 2018a). Anthocyanins are abundant in plants being responsible for the bright colours of fruits, flowers and vegetables and shield plants from environmental stresses (Landi *et al.,* 2015; Liang *et al.,* 2018; Bendokas *et al.,* 2020). The protective effects of anthocyanins have been attributed to their huge antioxidant prowess, their Cd chelating ability, ability to scavenge reactive oxygen species (ROS) and their ability to lower the gastrointestinal absorption of Cd (Brzóska *et al.,* 2016; Ereminas *et al.,* 2017). Anthocyanins can also stimulate the synthesis or activities of antioxidant enzymes, such as glutathione peroxidase, catalase (CAT) and superoxide dismutase (SOD) and inhibit the formation of ROS (Aboonabi & Singh, 2015; Reis *et al.,* 2016; Liobikas et al., 2016; Sun *et al.,* 2018; Zuo *et al*., 2019). The intake of anthocyanins have been shown to protect the livers, hearts, kidneys, brains and other tissues and organs of humans and animals due to their antioxidant activities and those of their metabolic derivatives (Liobikas *et al.,* 2016; Kalt *et al*., 2020; Bendokas *et al.,* 2020; Salehi *et al.,* 2020).

As noted by Asagba (2010) and Ezedom and Asagba (2016), animal studies on the effect of cadmium on aldehyde, xanthine, monoamine and sulphite oxidases are few, even though these enzymes are indispensable in the detoxification of toxins and other xenobiotics. In addition, despite the huge potentials of anthocyanins in ameliorating Cd toxicity, reports on the response of these enzymes in Cd-exposed rats treated with anthocyanins are scarce. Therefore, this study was designed to investigate the response of Oxidative Enzymes to *Hibiscus sabdarriffa* L. anthocyanins treatment in Cadmium-exposed Rats.

Methods

Plant Material

H. sabdariffa L. calyces were obtained from Warri Main Market, Warri South L.G.A., Delta State, and were left to dry at room temperature under continuous air-flow until constant weight. Plant material was authenticated by a Botanist in Delta State University, Abraka with voucher no. DEL/FOS/2015/2016/235.

Extraction and Purification of *H. sabdariffa* Anthocyanins

H. sabdariffa anthocyanins were obtained following the method of Hong and Wrolstad (1990) as described by Ologundudu *et al*. (2010). One (1) kg of *H. sabdariffa* calyces was pulverized and extracted with ten litres of 0.1% trifluoroacetic acid (TFA) for at 40 °C for twelve hours. This was followed by filtration with Whatman No. 1 filter paper. Thereafter, the filtrate was applied to silica-gel resin column (120 mesh) for fractionation of the different compounds in the extract. While sugars, acids and other water-soluble compounds flowed out when the column was washed with three litres of water, anthocyanins were absorbed. Anthocyanin pigments were thereafter eluted with 50% ethanol solution containing 0.1% TFA. The resulting eluate was dried at 40 °C under vacuum to obtain a concentrated eluate, which was then subjected to high-speed liquid chromatography (HPLC) to identify the purified anthocyanins and other active principles as described by Drust and Wrolstad (1990).

Experimental Animals

One hundred and twenty six (126) adult male wistar rats weighing 185±5.2g were used for the study. The rats were obtained from the animal house of the Faculty of Basic Medical

Sciences, Delta State University, Abraka. Prior to experiments, the rats were acclimated in a well-ventilated room maintained at 25 ± 2 ^{0}C with a 12h light/dark cycle for one week and were allowed access to clean water and feed *ad libitum*.

Ethical approval for the study was obtained from the Research Ethics Committee, of the Faculty of Science, Delta State University, Abraka, Delta State, Nigeria with approval number DEL/FOS/2016/2016/06. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Experimental Design and Treatment of animals

Experimental rats were divided into 18 groups with seven (7) rats in each group. Nine groups were used for 5 days acute toxicity study while the other nine groups were used for the 15 days chronic toxicity study.

Acute Toxicity study (5 days treatment)

Sixty-three rats were used for this study and were divided into six groups (A-F) with groups D- F having two sub groups as shown in Table 1 below.

Table 1. Acute toxicity study (5 days treatment)

A	**B**	**C**	**D (Anthocyanins)**		**E (Cd + Anthocyanin Pre-treatment)**		**F (Cd + Anthocyanin Post-treatment)**	
Ctrl	Cd (5mg/kg bw)	Aq Extract (1g/kg bw)	**D_1** Low Dose (1g/kg bw)	**D_2** High Dose (3g/kg bw)	**E_1** Low Dose (HSA (1g/kg b w) for five consecutive days before a single dose of Cd (5 mg/kg bw)	**E_2** High Dose HSA (3g/kg bw) for 5 consecutive days before a single dose of Cd (5 mg/kg bw)	**F_1** Low Dose A single dose of Cd (5mg/kg bw) on the first day then HSA (1g/kg bw) for 5 consecutive days	**F_2** High Dose A single dose of Cd (5mg/kg bw) on the first day then HSA (3g/kg bw) for 5 consecutive days

Sub-chronic Toxicity study (15 days treatment)

Sixty-three rats were used for this study and were divided into six groups (A-F) with groups D- F having two sub groups as shown in Table 2.

Table 2. Sub-chronic toxicity study (15 days treatment)

A	**B**	**C**	**D (Anthocyanins)**		**E (Cd + Anthocyanin Pre-treatment)**		**F (Cd + Anthocyanin Post-treatment)**	
Ctrl	Cd (3mg/kg b w) for 10 days	Aq Extract (1g/kg b w)	**D_1** Low Dose (1g/kg b w)	**D_2** High Dose (3g/kg b w)	**E_1** Low Dose HSA (1g/kg bw) for 15 consecutive days then Cd (3mg/kg bw) for five days	**E_2** High Dose HSA (3g/kg bw) for 15 consecutive days then Cd (3mg/kg bw) for five days	**F_1** Low Dose Cd (3mg/kg bw) for the first five consecutive days then HSA (1g/kg bw) for the remaining 15 days	**F_2** High Dose Cd (3mg/kg bw) for the first five consecutive days then HSA (3g/kg body weight) for the 15 ten days

H. sabdariffa anthocyanins and aqueous extract (in form of aqueous solution) were administered to the animals orally by orogastric tube. The doses of Cd and HSA used have previously been tested (Orororo *et al*., 2018). The animals were sacrificed twenty four hours

after the last treatment by cervical dislocation. The abdominal regions were opened exposing the heart, liver and other organs. Blood samples were obtained by cardiac puncture, placed in heparinized bottles and centrifuged at 3000g for 10 min. From each rat, the kidney, liver, testis, prostrate and epidydimis were obtained. They were weighed and 1 g portion of each homogenized in ice-cold saline and centrifuged at 5000g for 10 min. Sera and supernatants collected were stored frozen until used for analysis.

Assay for Aldehyde Oxidase (AO) Activity (E.C. 1.2.3.1)

The method of Omarov *et al.* (1999) was used for the determination of the activity of AO in the plasma and tissues of the rats and it is based on the decrease in absorbance at 600nm as benzaldehyde is oxidized to benzoate using 2, 6 –Dichloroindophenol as electron acceptor. Aldehyde oxidase activity was estimated using the following:

$$\frac{\Delta A \; X \; V_T}{\Sigma \; X \; V_S \; X \; X^1}$$

Where:
V_T = Total volume of reaction mixture
V_S = Volume of sample used
ΔA = Change in absorbance per minute at 420nm
X1 = Weight of tissue in reaction mixture
Σ = Molar extinction coefficient (19,100 m-1).

The activity of aldehyde oxidase was expressed in units per gramme tissue with one unit of the enzyme being the amount of the enzyme that produces one micromole of benzoate per minute.

Assay for Xanthine Oxidase (XO) Activity (E.C. 1.2.3.2)

The method of Beckman *et al.* (1989) was used for the estimation of the activity of Xanthine Oxidase in the plasma and tissues of the experimental and control rats.

The protocol is based on the quantification of the amount of uric acid formed as revealed by measurement of absorbance at 290 nm (A290). Exactly 50µm of hypoxanthine (as substrate), sample (1ml) (XO enzyme solution) and sodium phosphate buffer (pH 7.8, 50 mM) were mixed together in a cuvette (total volume 1.0-3.0mL). This mixture was incubated at 25 or 37°C while recording the time-dependent increase in the absorbance at 290 nm. XO activity in samples was determined by adding NAD+ to the reaction mixture. The enzymatic activity in each sample was calculated by use of the equation: units/mg protein = (ΔA/min×1000)/(1.22×104×mg mL-1 reaction mixture), with 1.22×104 M-1cm-1 taken as the molar absorption coefficient of uric acids. One unit of XO activity is defined as the velocity of the formation of 1 µmol uric acid min-1.

Assay for Sulphite Oxidase (SO) Activity (EC 1.8.3.1)

Sulphite oxidase activity in the plasma and tissue samples were determined by the reduction of ferricyanide according to the method described by Macleod *et al.* (1961). The assay is based on the reaction below:

$$Na_2SO_3 + O_2 + H_2O \longrightarrow Na_2SO_4 + H_2O_2$$

With ferricytochrome C as physiological electron acceptor, sulphite oxidase catalyses the oxidation of sulphite to sulphate and one unit of the enzyme is defined as the amount of the enzyme that reduces one micromole of ferricyanide per minute. This is calculated by the formular:

$$\frac{\Delta A \; X \; V_T}{\Sigma \; X \; V_S \; X \; X^1}$$

Where:
V_T = Total volume of reaction mixture
V_S = Volume of sample used
ΔA = Change in absorbance per minute at 420nm
X1 = Weight of tissue in reaction mixture
Σ = Molar extinction coefficient (1.02 X 104 m-1 cm-1).

Statistical Analysis

All the data obtained were subjected to statistical analysis of variance (ANOVA) and Least Significance Test (LSD) procedure using the SPSS software taking P-value of less than 0.05 ($p < 0.05$) as statistically significant. Results are expressed as Mean ± SD.

Results

The effect of *H. sabdariffa* anthocyanin on the activity of xanthine oxidase (XO) in the plasma and tissues of rats exposed to cadmium is shown in Table 3. The activity of XO in the plasma, epididymis, liver, kidney, prostrate and testes was significantly ($p<0.05$) reduced in rats maintained on Cd alone (Group B) compared to the control (Group A), rats administered aqueous extract of *H. sabdariffa* (Group C)and those maintained on high and low doses of *H. sabdariffa* anthocyanins (Groups D_1 and D_2) for both the acute and sub-chronic exposures. Rats exposed to aqueous extract and high and low doses of *H. sabdariffa* anthocyanins (Groups D_1 and D_2) recorded XO activities similar to the control for both acute and sub-chronic exposures in all the organs except in the plasma, where the administration of high dose of *H. sabdariffa* anthocyanins for the sub-chronic exposure significantly ($p<0.05$) increased XO activity relative to control. The administration of high and low doses of *H. sabdariffa* anthocyanins before and after Cd intoxication (Groups E_1-F_2) significantly ($p<0.05$) increased XO activity in the plasma and organs (except the testes) compared to rats maintained on Cd alone. This increase was to a level similar to the control mainly in the liver and prostrate for both the acute and sub-chronic exposures. Conversely, in the testes, pre and post treatment of Cd-exposed rats with high and low doses of HSA did not significantly ($p>0.05$) alter XO activity relative to rats exposed to Cd alone (Group B). This was observed for both modes of exposure.

The administration of low dose of *H. sabdariffa* anthocyanin before (Group E_1) and after (Group F_1) Cd exposure did not increase XO activity in the kidney compared to those administered alone (Group B) for the acute treatment, whereas a significant difference was observed for the sub-chronic treatment. However, rats given high dose anthocyanin before and after Cd exposure (Group E_2 and F_2) had significantly ($p<0.05$) higher XO activity than Cd only group for both treatment periods.

This study thus reveals that XO activity is significantly reduced by Cd exposure in the plasma, epididymis, liver, kidney, prostrate and testes, but pre and post-treatment of Cd-exposed rats with *H. sabdariffa* anthocyanins significantly increased XO activity in the plasma and the other organs studied except the testes.

Table 3. Effect of *H. sabdariffa* anthocyanin on the activity of Xanthine oxidase in the plasma and tissues of rats exposed to Cd

Groups	Xanthine Oxidase					
	Plasma	**Epididymis**	**Liver**	**Kidney**	**Prostrate**	**Testes**
Acute Exposure						
A	47.65 ±0.06[a]	67.12 ± 1.36[a]	62.62 ± 2.26[a]	39.62 ± 1.16[a]	43.34± 2.60[a]	43.14 ± 2.10[a]
B	24.52 ± 1.13[b]	41.32 ± 1.63[b]	44.23 ± 1.73[b]	25.55 ± 2.13[b]	34.25 ± 2.32[b]	33.55 ± 1.73[b]
C	46.45 ± 0.12[a]	66.24 ± 2.32[a]	61.56 ± 2.21[a]	37.12 ± 1.22[a]	40.05 ± 0.12[a]	41.52 ± 1.52[a]
D_1	48.22 ± 1.48[a]	62.32 ± 1.67[a]	61.27 ± 1.84[a]	38.33 ± 1.60[a]	40.12 ± 0.85[a]	40.16 ± 2.18[a]
D_2	50.03 ± 2.81[a]	65.07 ± 1.19[a]	60.35 ± 2.10[a]	37.12 ± 1.51[a]	42.37 ± 2.10[a]	41.06 ± 1.65[a]

E_1	30.12 ± 1.48^c	49.27 ± 2.58^c	60.22 ± 0.88^a	28.23 ± 2.60^b	39.55 ± 2.82^a	35.34 ± 3.02^b
E_2	31.13 ± 1.38^c	47.43 ± 2.68^c	57.34 ± 1.78^a	32.22 ± 1.11^c	39.22 ± 0.96^a	34.04 ± 1.88^b
F_1	42.32 ± 2.13^d	54.22 ± 2.63^d	59.38 ± 2.36^a	29.36 ± 2.40^b	34.22 ± 1.73^b	35.63 ± 2.63^b
F_2	45.14 ± 0.98^d	56.44 ± 2.12^d	59.46 ± 1.86^c	34.12 ± 1.41^c	40.23 ± 1.78^a	34.02 ± 1.88^b
Sub-chronic Exposure						
A	44.30 ± 0.62^a	68.23 ± 1.23^a	62.23 ± 1.14^a	40.22 ± 2.10^a	45.46 ± 2.03^a	43.65 ± 2.34^a
B	25.20 ± 0.93^b	39.22 ± 1.03^b	45.14 ± 1.89^b	24.52 ± 1.98^b	30.52 ± 1.24^b	32.05 ± 1.44^b
C	44.18 ± 0.34^a	65.12 ± 1.75^a	60.89 ± 1.11^a	39.05 ± 1.89^a	43.26 ± 1.24^a	42.24 ± 1.35^a
D_1	44.21 ± 1.05^a	63.42 ± 1.12^a	60.79 ± 1.66^a	39.23 ± 2.60^a	42.20 ± 1.56^a	41.50 ± 1.23^a
D_2	52.34 ± 2.56^c	64.24 ± 1.11^a	61.45 ± 1.90^a	38.26 ± 1.17^a	42.76 ± 1.76^a	41.78 ± 1.36^a
E_1	32.32 ± 0.99^d	52.34 ± 2.09^c	61.05 ± 1.09^a	30.16 ± 1.44^c	40.24 ± 1.55^a	37.47 ± 1.46^b
E_2	33.24 ± 1.24^d	51.30 ± 1.88^c	59.43 ± 1.23^a	31.02 ± 2.21^c	40.68 ± 1.68^a	36.34 ± 1.48^b
F_1	43.45 ± 1.32^a	53.00 ± 1.36^c	60.34 ± 1.65^a	30.65 ± 2.06^c	39.52 ± 1.34^a	36.80 ± 1.35^b
F_2	45.46 ± 0.86^a	55.10 ± 2.26^c	59.98 ± 1.66^a	32.24 ± 1.17^c	40.29 ± 1.34^a	35.06 ± 1.09^b

Note: Values are presented as mean ± standard deviation (SD) and as units/ ml (in plasma) and units/g tissue (in other tissues). Values on the column with different superscripts differ significantly ($P<0.05$) within the same treatment period/time. **Groups: A** (Control), **B** (Cd), **C** (Aqueous Extract), **D_1** (Low dose Anthocyanin), **D_2** (High Dose Anthocyanin), **E_1** (low dose Anthocyanin Pre-Cd), **E_2** (High Dose Anthocyanin Pre-Cd), **F_1** (Low dose Anthocyanin Post- Cd), **F_2** (High dose Anthocyanin Post-Cd)

Table 4 shows the effect of *H. sabdariffa* anthocyanins on sulphite oxidase (SO) activities in the plasma and tissues of Cd-exposed rats. In the plasma and all the tissues examined, sulphite oxidase activity was significantly ($p<0.05$) reduced in rats exposed to Cd alone (Group B) compared to the control animals for both the acute and sub-chronic exposure studies. SO activity recorded for rats maintained on high and low doses of *H. sabdariffa* anthocyanins alone (Group D_1 and D_2) were not significantly different from that recorded for the rats maintained on the aqueous extract alone (Group B) and the control (Group A) except in the prostrate (for the acute treatment) and in the liver where a significant difference was recorded in rats treated with high dose of HSA (Group D_2) for the sub-chronic treatment.

The administration of high and low doses of *H. sabdariffa* anthocyanins to rats before and after Cd exposure (Groups E_1-F_2) significantly ($p<0.05$) increased Sulphite oxidase activity in the plasma and all the organs assayed compared to rats exposed to Cd alone (Group B). This *H. sabdariffa* anthocyanins-induced increase was to a level comparable to that recorded for animals in the control group and those maintained on high and low doses of *H. sabdariffa* anthocyanins and *H. sabdariffa* aqueous extract alone in the epididymis (for the acute exposure) and in the kidney (for the sub-chronic exposure). This was also observed in the plasma by post-treatment of Cd-exposed rats with high dose of HSA (Group F_2).

The HSA pre and post treatment-induced increase in SO activity in Cd-exposed rats (relative to rats exposed to Cd alone) was observed to be dose dependent in the liver and kidney as well as the other organs, as rats given high dose of *H. sabdariffa* anthocyanins recorded higher SO activities (Group E_2 and F_2), although the values did not differ significantly ($p>0.05$) except in the plasma for the acute treatment.

These results indicate that Cd-induced reduction in SO activity in the plasma and organs was reverted by pre- and post treatment of Cd-exposed rats with *H. sabdariffa* anthocyanins, with high doses of HSA inducing higher activities of SO.

Table 4. Effect of *H. sabdariffa* anthocyanin on the activity of Sulphite Oxidase (SO) in the plasma and tissues of rats exposed to cadmium

Groups	Sulphite Oxidase					
	Plasma	**Epididymis**	**Liver**	**Kidney**	**Prostrate**	**Testes**
Acute Exposure						
A	22.34 ± 1.23^{a}	18.94 ± 1.36^{a}	104.58 ± 2.60^{a}	96.56 ± 2.23^{a}	75.80 ±2.23^{a}	40.24 ± 1.05^{a}
B	15.22 ± 1.09^{b}	15.30 ± 1.13^{b}	47.34 ± 1.78^{b}	55.15 ± 1.89^{b}	32.50 ± 1.21^{b}	21.50 ± 0.89^{b}
C	24.05 ± 1.24^{a}	19.08 ± 1.08^{a}	114.56 ± 2.51^{a}	95.45 ± 2.10^{a}	76.03 ± 2.10^{a}	40.32 ± 1.02^{a}
D_1	23.43 ± 1.18^{a}	20.13 ± 1.17^{a}	110.55 ± 2.30^{a}	92.44 ± 2.30^{a}	54.69 ± 1.88^{c}	40.10 ± 1.22^{a}
D_2	23.87 ± 2.01^{a}	20.13 ± 1.11^{a}	109.30 ± 2.71^{a}	93.46 ± 2.42^{a}	55.70 ± 1.72^{c}	40.00 ± 1.23^{a}
E_1	20.24 ± 1.80^{c}	20.33 ± 1.40^{a}	87.38 ± 1.98^{c}	77.09 ± 2.02^{c}	45.68 ± 1.28^{d}	33.24 ± 1.01^{c}
E_2	20.87 ± 1.30^{c}	20.88 ± 1.34^{a}	87.88 ± 1.66^{c}	78.44 ± 2.05^{c}	46.70 ± 1.86^{d}	33.40 ± 1.12^{c}
F_1	21.35 ± 1.54^{c}	19.90 ± 1.06^{a}	89.08 ± 2.04^{c}	79.06 ± 2.04^{c}	45.90 ± 1.74^{d}	34.68 ± 1.13^{c}
F_2	21.94 ± 0.88^{a}	20.14 ± 1.21^{a}	90.64 ± 2.45^{c}	79.90 ± 2.08^{c}	46.98 ± 1.70^{d}	34.90 ± 1.09^{c}
Sub-chronic Exposure						
A	20.18 ± 1.13^{a}	18.23 ± 1.45^{a}	100.84 ± 3.10^{a}	93.12 ± 2.30^{a}	79.86 ± 1.89^{a}	44.40 ± 1.00^{a}
B	13.20 ± 1.10^{b}	14.35 ± 1.34^{b}	57.44 ± 1.90^{b}	50.24 ± 1.26^{b}	30.34 ± 1.01^{b}	20.40 ± 0.65^{b}
C	19.44 ± 1.14^{a}	18.11 ± 1.23^{a}	102.60 ± 2.98^{a}	91.23 ± 2.34^{a}	79.06 ± 2.00^{a}	45.24 ± 1.04^{a}
D_1	18.48 ± 1.12^{a}	18.34 ± 1.71^{a}	102.23 ± 2.98^{a}	90.24 ± 2.07^{a}	78.04 ± 1.82^{a}	42.30 ± 1.45^{a}
D_2	18.77 ± 1.04^{a}	18.45 ±1.16^{a}	106.12 ± 2.87^{c}	91.60 ± 2.25^{a}	77.78 ±1.90^{a}	43.34 ± 1.57^{a}
E_1	15.10 ± 1.10^{c}	15.80±1.440^{c}	78.26 ± 2.01^{d}	89.04 ± 2.28^{a}	55.67 ± 1.12^{c}	38.14 ± 1.21^{c}
E_2	16.23 ± 1.20^{c}	16.30 ± 1.04^{c}	80.08 ± 2.11^{d}	89.98 ± 2.23^{a}	56.23 ± 1.16^{c}	38.28 ± 1.25^{c}
F_1	16.00 ± 1.15^{c}	16.01 ± 1.08^{c}	80.12 ± 2.08^{d}	89.04 ± 2.23^{a}	55.04 ± 1.19^{c}	38.60 ± 1.30^{c}
F_2	16.22 ± 1.06^{c}	16.95 ± 1.23^{c}	81.06 ± 2.15^{d}	89.10 ± 2.16^{a}	57.84 ± 1.21^{c}	38.96 ± 1.32^{c}

Note: Values are presented as mean ± standard deviation (SD) and as units/ml (in plasma) and units/g tissue (in other tissues). Values on the same column with different superscripts differ significantly (P<0.05) with the same treatment period/time. **Groups: A** (Control), **B** (Cd), **C** (Aqueous Extract), **D_1** (Low dose Anthocyanin), **D_2** (High Dose Anthocyanin), **E_1** (low dose Anthocyanin Pre-Cd), **E_2** (High Dose Anthocyanin Pre-Cd), **F_1** (Low dose Anthocyanin Post- Cd), **F_2** (High dose Anthocyanin Post-Cd)

Table 5 represents the effect of *H. sabdariffa* anthocyanins on aldehyde oxidase activities in the plasma and tissues of Cd-exposed rats.

The activity of AO was significantly ($p<0.05$) increased in the plasma of Cd-exposed rats after both the acute and sub-chronic exposures. Conversely, the activity of the enzyme was significantly ($p<0.05$) decreased in all the organs of Cd-exposed rats at the end of the sub-chronic exposure. Similarly, a significant ($p<0.05$) decrease in AO was observed in the epididymis (acute and chronic) and testes (acute and chronic exposure) of Cd-exposed rats (Group B), but a significant ($p<0.05$) increase was observed in the liver and kidney (acute) and no significant ($p>0.05$) change in prostrate (acute).

The plasma AO activity of rats treated with aqueous extract of *H. sabdariffa* (Group C), low dose of HSA (Group D_1) and high dose of HSA (Group D_2) was not significantly ($P>0.05$) different from the control after acute and chronic exposure studies. Similarly, the activity of the enzyme was not significantly ($p>0.05$) different from the control in the liver, kidney, prostrate and testes, but was significantly ($p<0.05$) decreased in the epididymis (Group C excluded) in rats administered aqueous extract of *H. sabdariffa* (Group C), low dose of HSA (Group D1) and high dose of HSA (Group D2) in both the acute and sub-chronic studies.

The activity of plasma AO was significantly ($P<0.05$) decreased in Cd-exposed rats (Group B) for both treatment periods after pre (Groups E_1 and E_2) and post (Groups F1 and F2) with low and high doses of anthocyanins as compared with rats exposed to Cd alone (Group B). Similarly, the activity of AO in the liver and kidney of Cd exposed rats (acute) was significantly ($P<0.05$) decreased in rats pre (Groups E_1 and E_2) and post (Groups F_1 and F_2) treated with low and high doses of anthocyanins relative to those exposed to Cd alone (Group B). Conversely, the activity of liver and kidney AO was significantly increased to levels comparable to control

in Cd-exposed (Chronic) rats after pre (Groups E_1 and E_2) and post (Groups F_1 and F_2) anthocyanins treatments. On the other hand, epididymis and testis AO activity was significantly ($p<0.05$) increased in rats for both mode of Cd exposure after pre (Groups E_1 and E_2) and post (Groups F_1 and F_2) treatment with both doses of anthocyanins as compared to rats exposed to Cd alone (Group B). However, no significant ($p>0.05$) change was observed in prostrate AO activity in Cd exposed (acute) rats after pre (Groups E_1 and E_2) and post (Groups F1 and F2) anthocyanins treatments. The prostrate AO activity (chronic study) was significantly ($p<0.05$) increased in rats pre (Groups E_1 and E_2) and post (Groups F_1 and F_2) treated with low and high doses of anthocyanins relative to Group B (Cd alone) rats.

The results obtained, indicate that pre and post treatment of Cd-exposed rats with HSA reversed Cd-induced changes in AO activities in the plasma and tissues of rats.

Table 5. Effect of *H. sabdariffa* anthocyanin on the activity of Aldehyde Oxidase (AO) in the plasma and tissues of rats exposed to cadmium

Groups	Aldehyde Oxidase (AO)					
	Plasma	**Epididymis**	**Liver**	**Kidney**	**Prostrate**	**Testes**
Acute Exposure						
A	12.26 ± 0.32^{a}	26.10 ± 0.45^{a}	103.63 ± 3.13^{a}	68.67 ± 2.10^{a}	21.23 ± 0.64^{a}	31.43 ± 1.12^{a}
B	24.02 ± 0.42^{b}	17.22 ± 0.23^{b}	120.06 ± 2.89^{b}	90.24 ± 2.34^{b}	19.99 ± 0.71^{a}	25.67 ± 1.00^{b}
C	11.28 ± 0.34^{a}	26.10 ± 0.47^{a}	102.03 ± 3.14^{a}	70.23 ± 2.11^{a}	20.89 ± 0.72^{a}	35.24 ± 1.02^{c}
D_1	10.28 ± 0.41^{a}	22.20 ± 0.41^{c}	100.01 ± 2.26^{a}	68.90 ± 2.02^{a}	21.00 ± 0.72^{a}	33.45 ± 1.13^{a}
D_2	11.01 ± 0.80^{a}	23.12 ± 0.42^{c}	101.10 ± 2.67^{a}	70.01 ± 2.78^{a}	21.45 ± 0.67^{a}	32.33 ± 1.05^{a}
E_1	18.79 ± 0.36^{d}	19.23 ± 0.51^{d}	110.23 ± 2.85^{c}	66.02 ± 1.98^{c}	20.01 ± 0.54^{a}	29.90 ± 1.10^{a}
E_2	18.60 ± 0.45^{d}	20.10 ± 0.43^{d}	111.24 ± 2.88^{c}	67.14 ± 1.56^{a}	20.14 ± 0.69^{a}	30.01 ± 1.16^{a}
F_1	18.23 ± 0.10^{d}	19.87 ± 0.54^{d}	110.67 ± 2.45^{c}	67.60 ± 1.77^{a}	20.04 ± 0.76^{a}	30.03 ± 1.32^{a}
F_2	17.04 ± 0.49^{e}	20.92 ± 0.62^{d}	113.00 ± 2.67^{c}	67.18 ± 1.34^{a}	20.18 ± 0.70^{a}	30.45 ± 1.36^{a}
Sub-chronic Exposure						
A	13.03 ± 0.60^{a}	26.56 ± 0.12^{a}	101.37 ± 3.13^{a}	72.34 ± 1.98^{a}	24.35 ± 0.49^{a}	34.32 ± 0.92^{a}
B	20.12 ± 0.38^{b}	17.67 ± 0.09^{b}	89.64 ± 2.89^{b}	52.20 ± 1.64^{b}	18.06 ± 0.51^{b}	20.50 ± 0.78^{b}
C	12.87 ± 0.62^{a}	26.80 ± 0.16^{a}	100.32 ± 3.14^{a}	73.34 ± 1.76^{a}	25.96 ± 0.50^{a}	36.20 ± 1.10^{a}
D_1	12.15 ± 0.61^{a}	22.78 ± 0.15^{c}	98.14 ± 2.26^{a}	68.90 ± 1.23^{a}	23.98 ± 0.52^{a}	35.53 ± 0.65^{a}
D_2	12.30 ± 0.53^{a}	22.10 ± 0.20^{c}	98.89 ± 2.67^{a}	70.01 ± 1.34^{a}	23.87 ± 0.64^{a}	34.24 ± 1.11^{a}
E_1	17.23 ± 0.40^{c}	20.14 ± 0.21^{c}	96.74 ± 2.85^{a}	60.32 ± 1.67^{c}	21.23 ± 0.54^{c}	25.67 ± 0.72^{c}
E_2	17.87 ± 0.42^{c}	20.67 ± 0.23^{c}	97.80 ± 2.88^{a}	61.45 ± 1.45^{c}	21.87 ± 0.48^{c}	26.12 ± 0.64^{c}
F_1	16.98 ± 0.66^{c}	20.69 ± 0.25^{c}	96.00 ± 2.45^{a}	62.03 ± 1.48^{c}	21.43 ± 0.46^{c}	27.76 ± 0.74^{c}
F_2	16.80 ± 0.45^{c}	20.23 ± 0.19^{c}	97.89 ± 2.67^{a}	62.84 ± 1.46^{c}	21.90 ± 0.56^{c}	27.86 ± 0.58^{c}

Note: Values are presented as mean $\pm$ standard deviation (SD) and as units/ml (in plasma) and units/g tissue (in other tissues). Values on the same column with different superscripts differ significantly ($P<0.05$) with the same treatment period/time. **Groups: A** (Control), **B** (Cd), **C** (Aqueous Extract), **D_1** (Low dose Anthocyanin), **D_2** (High Dose Anthocyanin), **E_1** (low dose Anthocyanin Pre-Cd), **E_2** (High Dose Anthocyanin Pre-Cd), **F_1** (Low dose Anthocyanin Post- Cd), **F_2** (High dose Anthocyanin Post-Cd)

Discussion

In this study, administration of Cd significant decreased the activity of xanthine oxidase (Table 3), sulphite oxidase (Table 4) and aldehyde oxidase (Table 5) in the plasma and tissues, but pre-treatment and post-treatment of Cd-exposed rats with *H. sabdariffa* anthocyanins reverted this effect. Given the fact that Cd induces oxidative stress, it is not surprising that these tissue oxidative enzymes were significantly reduced in Cd-treated animals. Ezedom and Asagba (2016) also reported similar changes in tissue oxidative enzymes in rats exposed to Cd through the food chain.

According to Asagba (2010), these oxidative enzymes (XO, SO, and AO) play important role in the biotransformation of xenobiotics. Kitamura *et al*. (2006) noted that AO and XO exhibits oxidative activity against aldehydes and different heterocyclic compounds. XO

functions principally in the catabolism of purines by facilitating the oxidation of hypoxanthine to xanthine and uric acid, while SO oxidizes endogenous sulphite gotten from the breakdown of sulphur-containing amino acids (Cohen *et al*., 1973). Thus, the alteration (reduction) observed in the activity of XO (Table 3), SO (Table 4) and AO (Table 5) in the plasma and tissues of rats treated with Cd would affect cellular metabolism in several ways such as in the detoxification of foreign molecules and in proper function of biomolecules that contain sulphur (e.g glutathione and metallothionein).

Several explanations for the Cd-induced inhibition of these enzymes (AO, XO and SO) have been provided by studies. Timbrell (2000), Islam *et al*. (2011), Zhang *et al*. (2013), and Asagba (2010) noted that it is connected to with the interaction of Cd with other essential metals at enzymes' active site leading to the displacement of the essential metals and Cd-induced formation of covalent bonds with sulphydryl and other groups necessary for the actions of the enzymes.

Pre-treatment and post-treatment of Cd-exposed rats with high and low doses of *H. sabdariffa* anthocyanins however increased the activities of AO, XO and SO in the plasma and tissues of rats. This again lends credence to the antioxidant powers of *H. sabdariffa* anthocyanins. Kowalczyk, (2003) reported the protective effects of anthocyanins from *Aronia melanocarpa* against Cd-induced oxidative stress and Hale *et al*. (2002) also showed that anthocyanins can quench oxygen radicals. This probably explains the increase in tissue oxidative enzymes observed when Cd-exposed rats were pre-treated and post-treated with graded doses of *H. sabdariffa* anthocyanins. This is also supported by the work of Al-Groom, and Al-Kubaisy (2016) where supplementation of diet of Cd-exposed rats with anthocyanin – rich red dye of HS counteracted the Cd-induced oxidative stress. There was no significant difference between pre-treatment and post-treatment with *H. sabdariffa* anthocyanins in the results obtained. This shows that the administered anthocyanin molecules were both protective and curative in ameliorating Cd toxicity probably due to their ability to decrease the bioavailability of Cd or to some other mechanisms, such as free radical quenching, electron transfer, radical addition, or radical recombination (Liangli *et al*., 2002).

Conclusions

Cd-induced alterations in the activities of the plasma and tissue oxidative enzymes is a confirmation of it reported toxic effects. Whereas, the positive ameliorative effects observed when Cd-exposed rats were pre and post treated with high and low doses of *H. sabdariffa* anthocyanins confirms the antioxidant potentials of the extract and shows that *H. sabdariffa* anthocyanins can be used in the treatment and prevention of Cd challenge in animal models with possible applications in human populations where Cd exposure is still inevitable.

List of Abbreviations

HSA: Hibiscus sabdariffa Anthocyanins
AO: Aldehyde Oxidase
XO: Xanthine Oxidase
SO: Sulphite Oxidase
SOD: Superoxide dismutase

Declarations

Ethics approval and consent to participate: Ethical approval for this research was obtained from the ethical committee of the Delta State University, Abraka, Nigeria and laboratory animals were handled in accordance with the laboratory principles of handling and care of experimental animals

Consent for publication: Not applicable.

Availability of data and material: Experimental data and materials employed in the development of this research report would be made available upon a reasonable request, but for the meantime, they are kept in the correspondence repository.

Competing interests: Not applicable.

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