ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Comparative Assessment of the Antioxidant Properties of *Hibiscus sabdarrifa* L Anthocyanins and its Aqueous Extract in Cadmium-exposed Rats

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ABSTRACT

Hibiscus sabdariffa L. anthocyanins (HSA) have been implicated in the reported antioxidant effects of *Hibiscus sabdariffa L.* aqueous extracts (HSAE) in Cd-exposed rats, but reports on the effects of HSA alone in Cd-exposed rats are scarce. The present study was therefore designed to compare the antioxidant properties of HAS and HSAE in rats following acute exposure to Cd. Thirty adult male wistar rats were randomized into five treatment groups: A: control, B: Cd, C: HSAE, D: HSA, E: HSAE Pre-Cd and F: HSA Pre-Cd. Exposure to Cd significantly decreased weight gain and reduced the level of GSH, the activities of CAT and SOD accompanied by increase in lipid peroxidation in rat liver compared to control. But pre-treatment with HSA and HSAE significantly ameliorated the changes induced by Cd. Pre-treatment with HSAE significantly increased GSH levels compared to pre-treatment with HSA, but HSA was more efficient in restoring the activities of CAT and SOD as well as in protecting tissue against Cd-induced peroxidation. The study thus shows that Cd toxicity results in depletion of tissue endogenous antioxidants, which can be ameliorated by the administration of HSA and HSAE. However, HSA seems to be a better ameliorator of Cd toxicity than HSAE.

Keywords: Antioxidants, Aqueous Extract, Oxidative Stress, Anthocyanins

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ISSN: 0975-8585

INTRODUCTION

Cadmium (Cd), like other heavy metals, has high density, occurs in the environment naturally in small quantity, but is an environmental and occupational pollutant due to its ubiquitous nature and increase in industrialization and human activities [1, 2, 3]. Through food, cigarette and occupational exposure, Cd gets into human system where its toxicity is manifested via induction and increase in tissue oxidative damage arising from increased oxidative stress [4].

Studies have shown the potential of various antioxidants in ameliorating Cd toxicity both *in vitro* and *in vivo* with many focusing on plant extracts which contain poly-phenolic compounds, flavoniods and anthocyanins [5, 6, 7]. *Hibiscus sabdariffa* L. (Hs) (Roselle), a member of the Malvaceae family, grown commonly in the tropical and other regions of the world is one plant whose extracts have been widely reported to have antioxidant effects against Cd and other heavy metals toxicities [8, 9, 10, 11, 12, 13, 14, 15, 16]. These researches have attributed the observed antioxidant properties of Hs extracts to its anthocyanins and other polyphenolic compounds, though studies on the effects of the specific components of the extracts on Cd toxicity are scarce. In addition, though the therapeutic purposes of anthocyanins have been proven, the specific and measurable pharmacological properties of isolated anthocyanin pigments in vitro and in vivo is just been explored. Authors have also noted that the exact roles of the anthocyanins in human health maintenance versus other phytochemicals in a complex plant extract have not been well understood with some suggesting that the antioxidant properties of anthocyanins is increased when administered as a mixture with other compounds [17].

The present study was therefore designed to compare the antioxidant properties of extracted Hs anthocyanins (HSA) and its aqueous extract (HSAE) in rats following acute exposure to Cd.

MATERIALS AND METHODS

Chemicals

The reagents used in this study were of analytical grade. Cadmium Chloride, methanol, trichloroacetic acid, acetonitrile and sodium chloride were purchased from Lobal Chemic Laboratory Regents and Fine Chemicals, Mumbai – India. 2,-thiobarbituric acid, Dichromate, acetic acid, adrenaline, and Ellman's reagent were gotten from BDH Chemical Company (Poole, England).

Plant Material

Fresh calyces of *H. Sabdariffa L.* were gotten from Warri Main Market, Warri South L.G.A., Delta State and were identified by a specialist in the Department of Botany, Delta State University, Abraka. Thereafter, they were dried under continuous air-flow maintained at room temperature until constant weight was achieved.

Preparation of aqueous extract

Aqueous extract of *H. sabdariffa calyces* was prepared as described by Iyare and Adegoke [18]. Dried *Hibiscus sabdariffa* calyx were boiled in distilled water for 15min.The boiled sample was allowed to cool and then filtered and the filtrate was evaporated to dryness at 40◦C in an oven to produce a dark red residue.

Extraction and Purification of H. sabdariffa anthocyanins

H. Sabdariffa L. calyces dried at room temperature under continuous air-flow till constant weight was achieved. Thereafter, anthocyanins were obtained following the method of Hong and Wrolstad [19] as described by Ologundudu et al., [20]. 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 was subjected to high-

speed liquid chromatography (HPLC) to identify the purified anthocyanins and other active principles as described by Drust and Wrolstad [21].

Experimental animals

Thirty adult male wistar rats weighing 185±5.2g were used for the study. The rats were obtained from the animal house of the University of Nigeria, Nsukka and were acclimatized for 1 week before the commencement of the experiment. Ethical approval was obtained from the Animal Ethics committee and the animals were handled according to standard laboratory and animal care guidelines in a spacious room with temperature of 25 ± 2 0C and 12h light/dark lightening system.

Experimental Design

Thirty adult male wistar rats (185±5.2g) were randomly divided into five treatment groups: A: control, B: Cd alone (a single dose of Cd 3mg/kg b wt), C: HSAE alone (aqueous extract 3mg/kg b wt), D: HSA alone (3mg/kg b wt), E: HSAE Pre-Cd (HSAE 3g/ kg b wt for five consecutive days before a single dose of Cd 3mg/kg b wt) and F: HSA Pre-Cd (HSA 3g/ kg b wt for five consecutive days before a single dose of Cd 3mg/kg b wt). The treatment lasted for five days at end of which, the animals were weighed and then sacrificed by cervical dislocation. From each rats the liver was obtained, weighed and 1 g portion homogenized in ice-cold saline (1:4, w/v) and centrifuged at 5000g for 10 min. Sera collected was stored frozen until used for biochemical analysis.

Biochemical Assays

The activity of Catalase in the liver of Cd-exposed rats was determined as described by Singha [22] with optical density measured at 570nm with a spectrophotometer. The method of Misra and Fridovich, [23] was employed in determining SOD activity in samples based on the inhibition of the autoxidation of adrenaline at pH 10.2 by superoxide dismutase. The increase in absorbance at 480nm was monitored every 30 seconds for 150 seconds and one unit of SOD activity was defined as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline to adenochrome during 1 minute. Reduced glutathione (GSH) was assayed according to the method of Beutler et al [24]. The optical density was measured at 412nm with GSH being proportional to the absorbance at this wavelength as estimated from a GSH standard curve. The level of Thiobarbituric acid reactive substances (TBARS) an indicator of lipid peroxidation was determined by the method of Varshney and Kale [25]. and computed with a molar extinction coefficient of 1.56 X 105 M-1 CM-1. Values of TBAS are reported in terms of malondialdehyde (MDA) and expressed as µmole MDA/g tissue.

Analysis of Data

Results are presented as Mean ± SD. Statistical analysis was performed using the Statistical Package for Social Sciences (SPSS) software. The one-way analysis of variance (ANOVA) was utilized in comparing level of significance difference between measured parameter using 0.05 as p value.

RESULTS AND DISCUSSION

The antioxidant effect of *H. sabdariffa* anthocyanin (HSA) and its aqueous extract (HSAE) on body weight gain of Cd-exposed rats is presented in Fig. 1. Exposure to Cd alone significantly decreased weight gain relative to control and rats maintained on HSA and HSAE. However pre-treatment of Cd-exposed rats with HSA and HSAE increased body weight gain compared to rats maintained on Cd alone. No significant difference was recorded in weight gain for rats maintained on HSA relative to those maintained on HSAE alone. Also, no significance difference was seen in body weight gain when Cd-exposed rats were pre-treated with HSA and compare to those pre-treated with HSAE.

Cd-induced reduction in body weight gain observed in this study agrees with earlier reports and supports the proven fact that Cd adversely influences ingested nutrients' digestion and metabolism [26, 10, 27, 28, 29]. HSA and HSAE-induced increase in body weight gain witnessed in this study is not unconnected with their nutritional and antioxidant properties [30, 31]. HSAE have been shown to contain significant amount of flavonoids, polyphenols, organic acids, anthocyanins and polysaccharides which supports its use traditionally as food and beverage all over the world [32, 33, 15]. Though no significant difference was observed in the

ISSN: 0975-8585

antioxidant effects of HSA and HSAE against Cd toxicity in terms of body weight gain (Fig 1), HSA administration induced better ameliorative effects against Cd-toxicity and may be attributed its higher content of anthocyanins compared to HSAE.

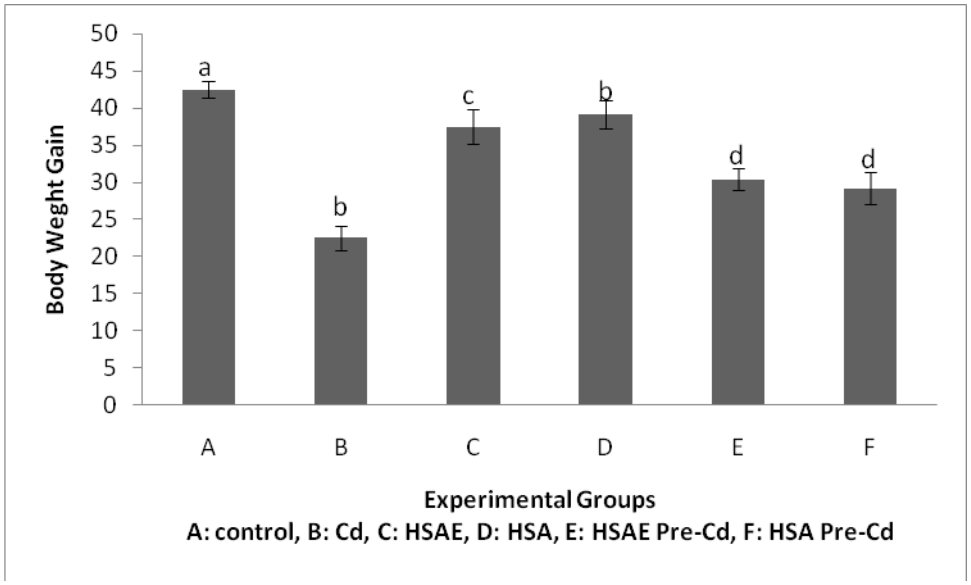

Fig 1: Antioxidant Effects of *H. sabdariffa* anthocyanin (HSA) and its aqueous extract (HSAE) on body weight gain of Cd-exposed rats. Values with different alphabetic superscripts differ significantly (P<0.05)

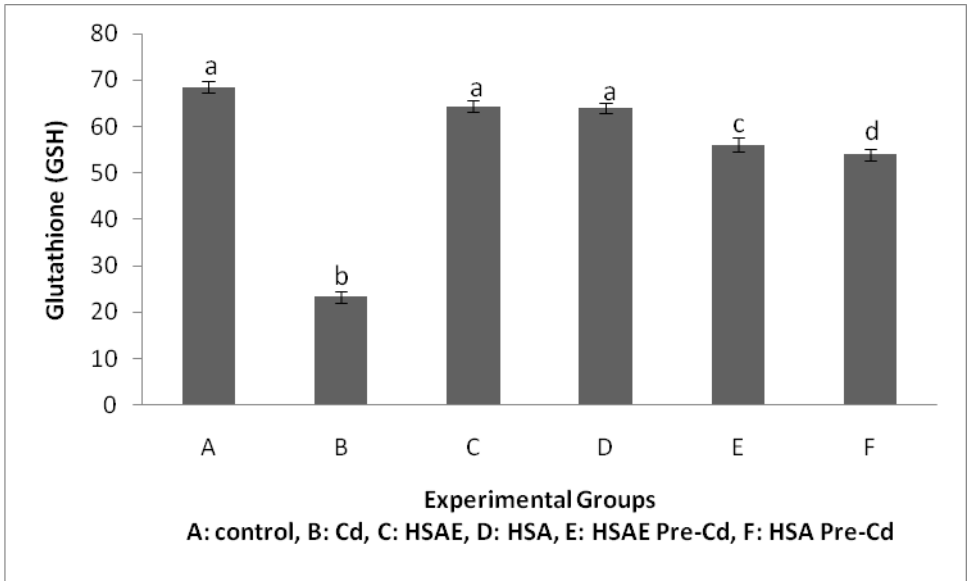

Fig 2: Antioxidant Effects of *H. sabdariffa* anthocyanin (HSA) and its aqueous extract (HSAE) on reduced glutathione (GSH) level in the liver of Cd-exposed rats. Values with different alphabetic superscripts differ significantly (P<0.05)

ISSN: 0975-8585

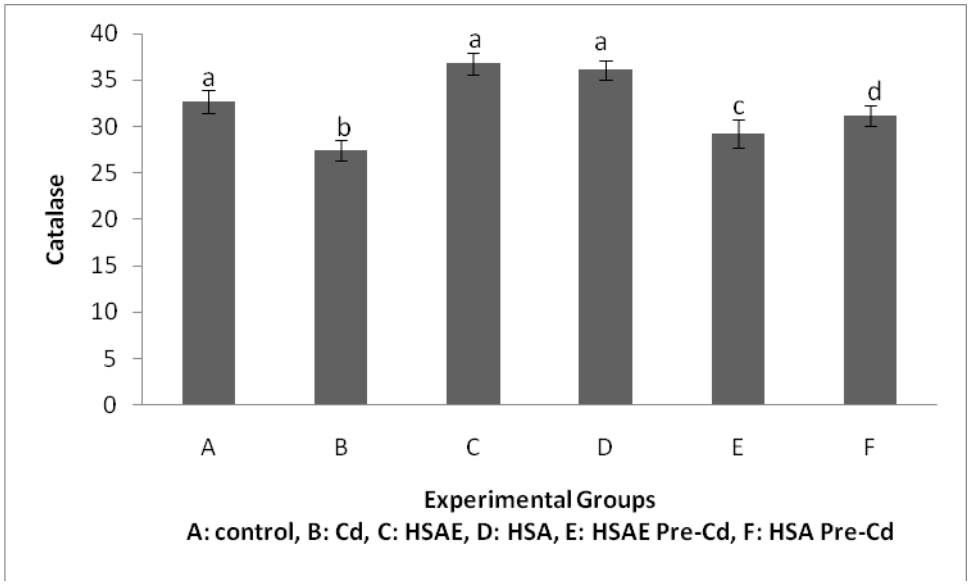

Fig 3: Antioxidant Effects of *H. sabdariffa* anthocyanin (HSA) and its aqueous extract (HSAE) on Catalase (CAT) activity in the liver of Cd-exposed rats. Values with different alphabetic superscripts differ significantly (P<0.05)

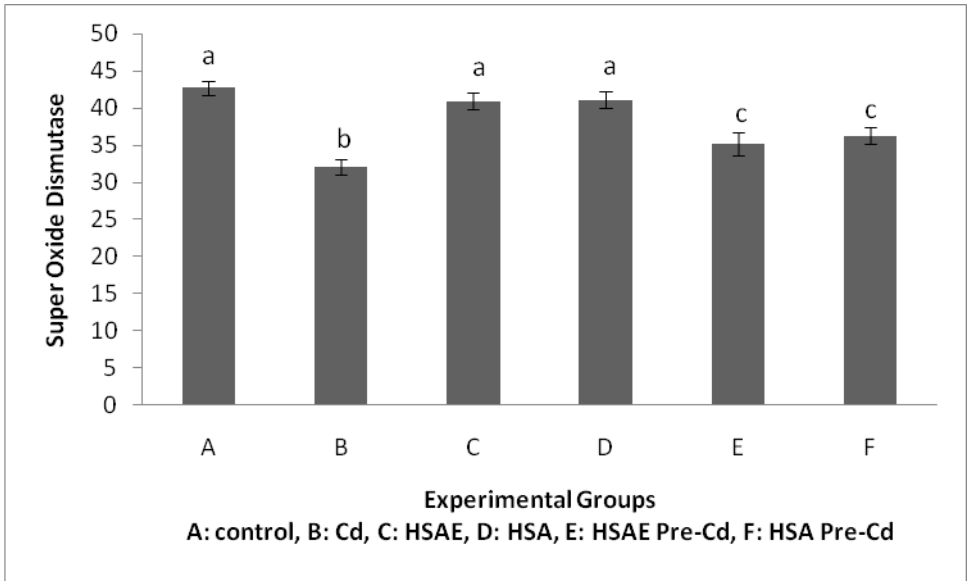

Fig 4: Antioxidant Effects of *H. sabdariffa* anthocyanin (HSA) and its aqueous extract (HSAE) on the activity of super oxide dismutase (SOD) activity in the liver of Cd-exposed rats. Values with different alphabetic superscripts differ significantly (P<0.05)

ISSN: 0975-8585

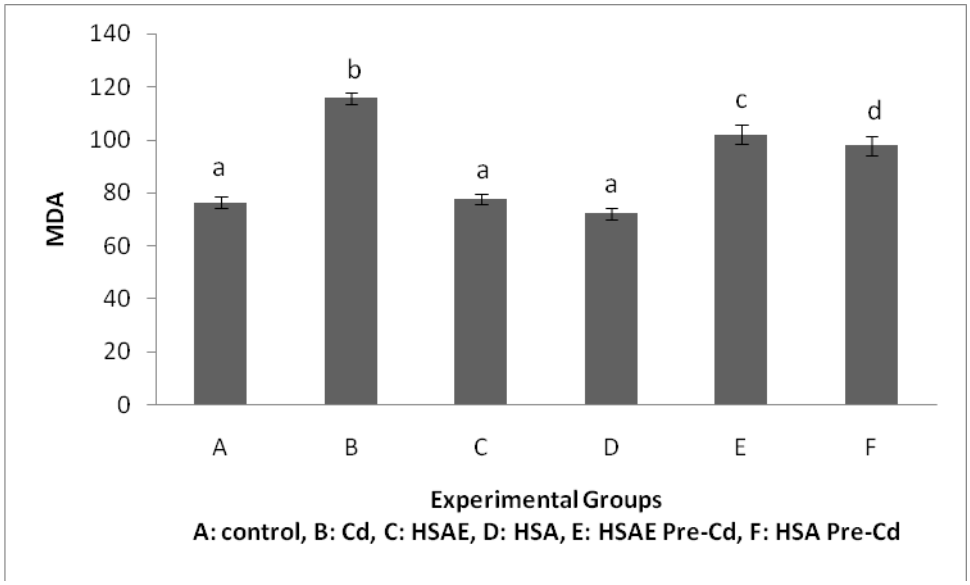

Fig 5: Antioxidant Effects of *H. sabdariffa* anthocyanin (HSA) and its aqueous extract (HSAE) on the level of lipid peroxidation (MDA) in the liver of Cd-exposed rats. Values with different alphabetic superscripts differ significantly (P<0.05)

Antioxidant effects of HSA and HSAE on GSH and tissue oxidative enzymes is shown in Fig 2-4, while their effect on tissue lipid peroxidation is shown in Fig. 5. Exposure to Cd alone (Group B) significantly reduced the level of GSH (Fig. 2), the activities of CAT (Fig. 3) and SOD (Fig 4) accompanied by increase in peroxidation (Fig. 5) in rat liver compared to control and the other treatment groups.

The Cd-induced depletion of endogenous non enzymatic (GSH) and enzymatic (CAT and SOD) antioxidants observed is in consonance with the reported ability of Cd to induce oxidative stress by causing increase in the generation of reactive oxygen species and free radicals [34, 35, 5, 29, 36]. This results in increase peroxidation of tissue membrane lipids which is also witnessed in this study.

As shown in Fig 2-5, the level of GSH and tissue lipid peroxidation and the activities of CAT and SOD were not significantly affected by treatment of rats with HSAE and HAS alone relative to control, but when Cd-exposed rats were pre-treated with HSAE and HSA (Groups E and F), a significant increase in the level of GSH and the activities of CAT and SOD accompanied by a significant reduction in tissue lipid peroxidation was recorded relative to rats maintained on Cd alone (Group B). Again, this is in consonance with the reported abilities of HS extracts to offer protection against Cd-induced oxidative stress [37, 38, 39] and reinforces the claim that HSA and HSAE have antioxidant properties that can effectively ameliorate Cd toxicity. Comparing the antioxidant effects of HSA and HSAE showed that pre-treatment with HSAE significantly increased GSH levels compared to pre-treatment with HSA, but HSA was more efficient in restoring the activities of CAT and SOD as well as in protecting tissue against Cd-induced lipid peroxidation (Fig. 5).

CONCLUSION

This study has shown that Cd toxicity results in depletion of tissue endogenous antioxidants but this can be ameliorated by the administration of HSA and HSAE. However, HSA seems to be a better ameliorator of Cd toxicity than HSAE.

Declaration: This manuscript is original and is not published or communicated for publication elsewhere either in part or full.

Competing interests*:* "The authors declare that they have no competing interests"

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