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Original Article

Treatment with *Hibiscus sabdarrifa* L Anthocyanins Improve Hematological Parameters in Rats Exposed to Cadmium

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Abstract

Background and objectives: Cadmium (Cd) is toxic to blood cells and other tissues of the body. This study examined the influence of *Hibiscus sabdarrifa* anthocyanins (HSA) pretreatment on selected blood parameters in rats administered Cd.

Methods: Forty Wistar rats were randomized into eight groups with five rats in each group. The rats were handled in two experimental periods: a five-day acute study and 15-day chronic toxicity study. The experimental groups were the control, Cd, HSA and HSA+Cd groups.

Results: Compared with the healthy control, Cd administration significantly increased the counts of white blood cells (WBC), but decreased red blood cells (RBC), platelets, packed cell volume (PCV), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH) in rats ($p < 0.05$ for all). In contrast, treatment with HSA significantly reduced the hematological toxicity of Cd in rats by decreasing the counts of WBC, but increasing RBC, PCV, Platelets, Hb, MCV, MCHC, and MCH values in rats.

Conclusions: The results indicate that HSA treatment ameliorates the Cd-caused hematological toxicity in rats and may be valuable for intervention of Cd poisoning.

Introduction

Cadmium (Cd) application in industrials in many developing countries has caused environmental pollution, leading to acute and chronic Cd poisoning.[1] According to Okorie *et al*.,[2] Cd (and its compounds) is presently regarded as one of the major hazardous pollutants in the world. Cd remains in the environment for a long time and has highly toxic effects. Cd can be inhaled and ingested by the general population, especially workers in industrials, and its accumulation can cause acute and chronic intoxications. Ingestion of Cd-contaminated agricultural products is a major route for human intoxications.[3] Cd presents in cigarettes, batteries, paints and other industrial products.[4]

After ingestion or inhalation, Cd mainly accumulates in the liver, kidneys, and reproductive organs and causes its toxicity. In the liver, Cd causes hepatocyte swelling, necrosis, and degeneration.[5–7] Cd can induce strong oxidative stress and membrane lipid peroxidation that mediate its toxicity in different organs. These effects on the liver and those on other organs of the body have been attributed to Cd-induced oxidative stress, which has been reported as the major means of Cd toxicity.[8–11] Particularly, Cd-toxicity can damage the function and structure of bone and blood cells and inhibit their functional development.[12,13] After entering the blood circulation, Cd can bind to red blood cell membranes,

Keywords: Cadmium; Hematological parameters; *Hibiscus sabdarrifa* anthocyanins; Oxidative stress..

Abbreviations: Cd, Cadmium; Hb, hemoglobin; HSA, *Hibiscus sabdarrifa* anthocyanins; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PCV, packed cell volume; RBC, red blood cells; WBC, white blood cells.

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Table 1. Experimental design

Control	Cd	HSA	Cd+HSA
Acute study – 5 days			
Control (food and water only)	Cd (3 mg/kg body weight)	HSA (3 g/kg body weight)	HSA Pre-Cd High Dose; HSA (3 g/kg body weight) for 5 consecutive days and a single dose of Cd (5 mg/kg body weight)
Chronic study – 15 days			
Control (feed and water only)	Cd (3 mg/kg body weight)	HSA (3 g/kg body weight)	HSA Pre-Cd (HSA 3 g/ kg body weight for 10 consecutive days and Cd 3 mg/kg body weight for 5 days)

Cd, Cadmium; HSA, *Hibiscus sabdarrifa* anthocyanins.

where it triggers the production of metallothioneins and increases the generation of reactive oxygen species, thereby damaging the antioxidant defense system and red blood cells.[14,15]

Previous studies have shown that natural antioxidants can counteract the Cd-induced oxidative stress and in this regard, plant pigments such as anthocyanins and other polyphenols have shown great potentials.[16,17] Blood parameters are good indicators of the physiological and nutritional status of animals and reflect the changes in the metabolic processes.[18] These parameters can also measure how well nutrients and other food additives are metabolized by living organisms, and the relationship between chemicals, drugs, and various plant extracts and blood functions.[19,20]

Hibiscus sabdariffa L. (Hs) (*Malvaceae*) is also known as roselle and is an annual, herbaceous, subshrub with typically red stems and flowers. The plant is largely grown in the tropics and subtropics, such as Thailand, Sudan, West India, Egypt, Mexico and China. In Nigeria, Thailand, Mexico, Egypt and Sudan, roselle juice, made by water-extraction from the calyces, is a soft drink loved and consumed by many people.[17] Given its excellent safety profile, this study aimed to investigate the influence of *Hibiscus sabdarrifa* anthocyanins (HSA) pretreatment on selected blood parameters in rats administered Cd.

Materials and methods

Chemicals and reagents

All chemicals and reagents utilized in the experiments were of analytical grade and complied with standard quality specifications.

Plant material procurement and processing

Fresh Hibiscus sabdariffa L. calyces were procured from local markets within Warri, Delta State. The calyces were air-dried at ambient temperature, ensuring adequate airflow to maintain their integrity.

Anthocyanin extraction and purification

Hibiscus sabdariffa anthocyanins (HSA) were extracted and purified following established methodologies detailed in the literature.[17,21,22]

Animal selection and husbandry

Forty male Wistar rats, with body weights ranging from 188 to 192 g, were selected for the study. Prior to the initiation of experimental procedures, the rats underwent a seven-day acclimatization period in a controlled environment maintained at 25 ± 2°C with a 12-hour light-dark cycle. The rats were housed in standard laboratory cages and cared for following accepted laboratory animal care protocols.

Ethical considerations

The ethical approval for the research was granted by the Ethics Committee of the Faculty of Science at Delta State University, Abraka (Approval No. SCI/2015/7b). All procedures were designed to adhere strictly to international and national standards for animal welfare (See Supplementary File 1).

Experimental design and dosage regimen

The rats were assigned to eight experimental groups, each containing five animals. The study comprised two phases: an acute phase spanning five days and a sub-chronic phase lasting fifteen days. The treatment regimens for each group were administered orally each morning, as outlined in Table 1. The groups consisted of a control group, a Cd group, an HSA group, and an HSA+Cd group, with each having corresponding sub-groups for the acute and sub-chronic phases of the study.

Animal sacrifice and sample collection

Upon completion of the respective experimental periods, the rats were humanely euthanized. Blood samples were then collected for subsequent hematological analyses.

Hematological analysis

Hematological parameters, including platelet count, hemoglobin concentration (Hb), red blood cell count (RBC), white blood cell count (WBC), packed cell volume (PCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (MCH), and mean corpuscular volume (MCV), were determined using an automated hematology analyzer.

Statistical analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) software. Group differences were assessed using repeated measures analysis of variance (ANOVA), with a *p*-value of

Table 2. Influence of *H sabdariffa* L Anthocyanin on hematological parameters/indices of rats exposed to Cd

Exposure	Parameters	Groups			
		Control	Cd	HSA	Cd+HSA
Acute Exposure	WBC (10^4/mm^3)	449 ± 0.16	785± 0.17*	453± 0.22	638± 0.29*#
	RBC (10^6/mm^3)	450 ±0.38	203± 0.29*	518± 0.46*	360 ±0.23*#
	Hb (g/dl)	10.08±0.19	05.02±0.27*	12.01 ± 0.42	08.25± 0.47*#
	PLT (10^3/mm^3)	22.01±0.11	15.22± 0.09*	41.11± 0.38*	24.45± 0.22#
	PCV (%)	40.92±0.10	32.53±0.17*	44.60± 0.22	39.44± 0.29#
	MCV (fL)	69.14 ± 2.56	63.17 ± 1.45*	69.74 ± 1.36	65.11 ± 1.12*
	MCH (pg)	19.10 ± 0.62	20.13 ± 1.23*	17.34 ± 1.24*	18.05 ± 1.42#
	MCHC (g/L)	323.42 ± 4.16	301.10 ± 3.22*	327.45 ± 4.01	311.35 ± 3.66*#
Chronic Exposure	WBC (10^4/mm^3)	438 ±0.12	780±0.32*	444±0.20	530±0.19*#
	RBC (10^6/mm^3)	446± 5.45	233±5.32*	523± 4.47*	410± 4.29*#
	Hb (g/dl)	11.12± 0.19	04.92±0.27*	11.89± 0.42	09.95±0.47*#
	PLT (10^3/mm^3)	24.0± 0.11	14.8± 0.09*	43.3±0.37*	25.4± 0.22#
	PCV (%)	44.2± 0.24	30.8± 0.27*	43.8±0.23	38.5±0.32*#
	MCV (fL)	76.12 ± 1.26	68.34 ± 2.53*	75.19 ± 1.84	70.22 ± 2.42*#
	MCH (pg)	19.75 ± 2.34	15.34 ± 2.31*	19.45 ± 2.41	17.51 ± 2.23*#
	MCHC (g/L)	314.22 ± 3.62	299.15 ± 3.26*	315.15 ± 3.12	308.45 ± 3.36*#

Values are shown as mean standard deviation of each group (n = 3) of rats. *shows statistical significance at $p < 0.05$ vs. the control; while *#shows statistical significance at $p < 0.05$ vs. the Cd group. Cd, Cadmium; Hb, hemoglobin; HSA, Hibiscus sabdarrifa anthocyanins; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; PCV, packed cell volume; PLT, platelet ; RBC, red blood cells; WBC, white blood cells.

less than 0.05 considered to indicate statistical significance. Data are presented as mean ± standard deviation (SD)

Results

Compared with the controls, pretreatment with HSA did not significantly change the values of any hematological parameter, except for significantly increasing the values of RBC and platelets in both models of rats (Table 2). Secondly, administration with Cd caused acute hematological toxicity by significantly increasing the counts of WBC, but decreasing the values of MCH, PCV, platelets, MCV, RBC, Hb, and MCHC in rats.

Pretreatment with HSA significantly mitigated the Cd-changed values of these hematological parameters in both models of rats, except for insignificantly increasing MCH values in the acute toxicity model of rats.

Discussion

Hematological parameters can be used for evaluation of nutritional status of individuals and toxicity profile of drugs, chemicals, and food products.[9,23] In this study, we found that Cd administration significantly increased the counts of WBC, but decreased the values of Hb, platelets, RBC, PCV, MCV, MCH, and MCHC in rats, consistent with previous observations.[6,9,24,25] Similarly, Cd administration has been reported to reduce the values of Hb, PCV, and platelets in rats,[10] although this study did not detect the reduced values of MCH and MCV in rats. However, the reduced values of MCV and MCHC by Cd were also reported by İhsan *et al*.[26]

Mechanistically, the Cd-induced anaemia may be mediated by intravascular hemolysis, direct damage of Cd to erythrocytes, and iron depletion in the liver, spleen and other tissues.[27,28] In addition, Ladokun *et al*.,[29] have noted that the decreased counts of RBC can be explained by a corresponding decrease in MCV and MCH, which determine the size of RBCs. Thus, the decreased values of MCV and MCH by Cd exposure may have resulted in a decrease in RBC counts and Hg contents in rats. The results also agree with a previous study that found that Cd induced microcytic hypochromic anemia in rats.[30]

The insignificant changes in PCV, WBC, and Hb values and significant changes in RBC and platelets values caused by administration of HSA are similar to some previous reports, but not in agreement with others. Olatunji *et al*.[31] and Famurewa *et al*.[32] indicated that treatment with *H sabdariffa calyx* aqueous extract failed to significantly change the values of Hb, RBC, platelets, and PCV in rats. On the other hand, Ejere *et al*.[33] reported that treatment with *H sabdariffa* calyces significantly increased the values of these parameters in rats. Nevertheless, we found that treatment with HSA significantly increased the counts of RBC, pointing to the ability of HSA to enhance the RBC development. This, together with increased values of PCV and Hb, suggests that HSA has blood boosting properties. It is possible that administration of HSA may accelerate RBC development or limit RBC destruction in rats. The therapeutic effect of HSA indicates that HSA may have potentials as a health promoting molecule in ameliorating Cd toxicity.

Our findings were also consistent with previous reports that treatment with HS extracts elevates the levels of Hb, RBC, and

PCV.[34,35] Interestingly, these studies have highlighted the importance of antioxidant anthocyanins, flavonoids, and polyphenolic acid in the medicinal effects of *Hibiscus sabdariffa*.[36] Therefore, our findings provide new evidence to demonstrate that HSA has potent antioxidant activity to mitigate the Cd-hematologic toxicity.

Future directions

The development of red and white blood cells is regulated with great precision in healthy humans, but the number of leukocytes greatly rapidly increases during infection. Chronic Cd intoxication commonly induces anemia, which may be due to intravascular hemolysis or to the direct damaging effect of Cd on erythrocytes. The exact mechanism underlying the hematologic toxicity of Cd remains to be examined. We are also interested in further investigating how HSA treatment significantly increases WBC counts in rats and whether HSA treatment can also promote the development maturation of other types of blood cells.

Conclusions

In this study, our data indicated that Cd administration significantly increased the counts of WBC, but decreased the values of other hematologic parameters we tested in rats, and pretreatment with HSA significantly increased RBC and Hb values in healthy rats and mitigated the Cd-altered hematological parameters we tested in rats. These results suggest that HSA may be valuable for the intervention of Cd-mediated hematologic toxicity. Our findings also indicated that anthocyanins had potent antioxidant activity against oxidative stress in the pathogenesis of many diseases.

Supporting information

Supplementary material for this article is available at https://doi.org/10.14218/JERP.2022.00003.

Supplementary File 1. ARRIVE Checklist.

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Conflict of interest

The authors declare no competing interests.

Author contributions

OOC and ASO designed the experiment, OOC carried out the laboratory work, OOC and ASO wrote and edited the article.

Ethical statement

Ethical approval for the study was obtained from the Ethic Committee of the Faculty of Science, Delta State University, Abraka (SCI/2015/7b). All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

Data sharing statement

The data used to support the findings of this study are available from the corresponding author

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