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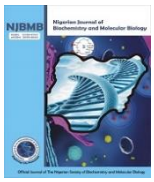

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Research Article

Cadmium Induced Hepato-Nephro Toxicity in Rat Model: Protective and Curative Influence of *Hibiscus sabdarrifa* L. Anthocyanins

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ABSTRACT

The liver and kidney are prime targets of cadmium (Cd) toxicity and the search for antidotes of Cd-induced hepato-renal toxicity is an important area of research. This research was designed to assess the impact of anthocyanins from Hibiscus sabdarrifa L. on cadmium-induced hepato-nephro toxicity in rats following acute and chronic Cd exposure. Fifty adult male Wistar rats were shared into ten (10) groups (A-E): Naive control, Cd control, anthocyanins control, Cd (3g/body weight) + anthocyanins (/3mg/kg body weight) pre-treatment and Cd+anthocyanins post-treatment. Five groups were used for 5days acute toxicity study while the other five groups were used for the 15days chronic toxicity study. The organ/body weight ratio for the liver and kidney were considerably ($p<0.05$) reduced in rats after acute and sub-chronic exposure to Cd The administration of Cd to rats in both modes of exposure significantly ($p<0.05$) increased AST activity in serum with a corresponding decrease in the liver, but the changes were improved by pre and post treatment of Cd-exposed rats with HSA. Similarly, acute and chronic exposure to Cd significantly increased ALT activity in serum with a corresponding decrease in the liver. Pre-treatment and post-treatment of Cd-exposed rats with HSA considerably lowered serum creatinine and urea equated to rats exposedto Cd alone. The results showed that administration of cadmium to rats altered liver and kidney function indices. However, pre-treatment and post-treatment of Cd-exposed rats with H. sabdariffa anthocyanins meaningfully reversed these effects indicating that HS anthocyanins can ameliorate Cd-induced hepato-renal toxicity in Wistar rats.

Keywords: *Cadmium, Liver, Kidney, Anthocyanins*

INTRODUCTION

Heavy metal toxicity is an old environmental problem that has continued till today (Satarug *et al*., 2017). Among heavy metals of concern is Cadmium (Cd), which is a non-essential element (WHO, 2011). Cd is commonly found together in ores with other metals such as zinc, lead and copper (Elias *et al.*, 2013; Song *et al.*, 2015). Arising from increased industrial, volcanic and agricultural activities and its non-bio-degradable nature, Cd is present in the environment in toxic levels, from where humans are generally exposed through inhalation and ingestion (WHO, 2011; Nordberg *et al*., 2011; Satarug *et al*., 2017). Its toxicity is mediated through a number of different routes such as interactions between Cd and essential metals and the oxidative stress caused by Cd exposure (Vesey, 2010; Orororo *et al*., 2018).

The liver and kidneys, which are in charge for the metabolism of almost all xenobiotics in the body, are usually affected by the toxic effects of Cd were it induces oxidative stress, disrupts the normal function of essential metals and causes damages (Vesey, 2010; Hayati *et al*., 2017; Imafidon *et al*., 2018; Ndubuisi *et al*., 2020). In the liver, Cd-toxicity is manifested by swelling of hepatocytes, fatty changes,

focal necrosis, destruction of ribosomes and lysosomes and impairment in the liver function biomarkers such as activities of alanine aminotransferase (ALT), aspartate aminotraerase (AST) and alkaline phosphatase in the plasma (El-Sokkary and Awadalla, 2011). The kidneys are crucial in the control of electrolytes and intracellular fluid volume, as well as the elimination of metabolic waste from the body. As a result, whole body homeostasis is dependent on the kidneys' functional integrity (Kamal, 2010). Any drug that is harmful to the kidney would have a negative impact on overall body metabolism (Kolawole *et al*., 2014). Cd-induced nephro-toxicity results in deregulated blood pressure, osteoporosis, damage of tubules, diabetes complication, decreased reabsorption of low molecular weight molecules and eventually renal failure (Messner *et al*., 2010; Prozialeck and Edwards 2012; Chakraborty *et al.*, 2013; Wallin *et al.*, 2014; Satarug *et al.*, 2017).

Given the many important functions of the liver and kidneys, and the reported effects of cadmium toxicity on these organs, the search for antidotes of Cd-induced hepatonephro-toxicity is an important area of research. Edible plants when taken at adequate levels make vitamins and essential metals available to the body, which can decrease the risks of Cd toxicity. Plant nutrients, dietary proteins, and phytochemicals such as anthocyanins have been shown to have beneficial benefits such as antioxidant properties against Cd poisoning (Zhai *et al*., 2015).Many substances including plant pigments, products and extracts such as ethanolic leaf extract of *Calycopteris floribunda* (Rajasekaran and Periasamy, 2012), garlic extract (Ayakeme *et al*., 2012), polyphenol-rich extract of the leaves of *Vernonia amygdalina* (PEVA) (Imafidon *et al*., 2015), rosemary leaf extract (Sakr *et al*., 2015) have been assessed for their potential antioxidant effects against Cd-induced toxicities. They are readily available and have fewer side effects compared to chelation therapy in treating Cd toxicity (Monachese *et al.*, 2012). In the past, chelating compounds such as calcium disodium versenate, dimercaprol and mesomercaptosuccinic acid have been used in treating Cd intoxications (Shaikh *et al.*, 1999;). However, some safety and efficacy concerns about these chelators have been reported such that no chelating therapy in ameliorating Cd toxicity has so far been approved for clinical use (McCarty, 2012). For example, meso-2,3-dimercaptosuccinic acid (DMSA) which have protective effects against Cd/Pb toxicity, causes loss of appetite, nausea and diarrhea (Liebelt *et al.*, 1994).

Anthocyanins are flavonoids produced by plants to protect them from cold temperatures, drought, UV light and other environmental stress factors, which cause damage to cells and tissues (Chalker-Scott, 1999). They are glycosylated polyhydroxy and polymethoxy derivatives of flavilium salts with characteristic C3 – C6 – C3 carbon structure and flavylium cations forming ability (Mazza, 2007). They have been shown to possess anti-inflammatory, antioxidative and metal ion chelating properties (Wang, 2000). *Hibiscus sabdariffa L.* (Hs), often known as roselle, is an annual herbaceous subshrub with generally red stems and blooms that have different names in different regions of the world and flourish in a variety of environments (Mortn, 2010). Ajiboye *et al*. (2011) reported that Roselle calyx extract is a good source of antioxidants due to its anthocyanin content. Studies on the effect of Hs extracts on Cd-induced toxicity have been done (Asagba, 2010; Alzubade, 2014; Kolawole *et al*., 2014) but studies using specific components of Hs extracts are unreported. The current research was thus planned to look into the outcome of anthocyanins from *Hibiscus sabdarrifa* L. on cadmium-induced hepato-nephrotoxicity in rats.

MATERIALS AND METHODS

H. sabdariffa anthocyanins were isolated and purified from dried *H. sabdariffa* L. calyces using conventional techniques detailed in our earlier research (Orororo *et al*., 2018). The presence of malvidin-3-O-glucoside, delphinidin-3-monoglucoside, cyanidin-3-monoglucoside, and petunidin-3-monoglucoside was shown by HPLC analysis (HPLC Cyber Lab Corporation, USA) of the extract.

Experimental animals

Fifty (50) adult male Wistar rats weighing 185 ± 5.2 g were employed in the research. The rats were gotten from the University of Nigeria animal house, Nsukka. Before the experiment commenced, the rats were kept in spacious rooms with temperature of 25 ± 2 °C and 12h light/dark lightening system to acclimatize for one week and were maintained on clean water and grower's mash (Hybrid Feeds, Ibadan, Oyo State, Nigeria) *ad libitum*.

Animal treatment and experiment design

The experimental rats were split into ten (10) groups of five (5) rats each. As indicated in Tables 1 and 2, five groups were utilized for the 5-day acute toxicity research, while the remaining five groups were used for the 15-day chronic toxicity study:

Table 1: Experimental design for 5 days acute toxicity study

A (Naïve Control)	B (Cd Control)	C (Anthocyanin Control)	D (Cd + Anthocyanin Pre-treatment)	E (Cd + Anthocyanin Post-treatment)
Feed and water only	single dose of cadmium (3mg/kg body weight)	anthocyanin extract (3g/kg body weight) for five days	anthocyanin (3g/kg body weight) for five consecutive days before a single dose of Cd (3mg/kg body weight)	a single dose of Cd (3mg/kg body weight) on the first day then Anthocyanin (3g/kg body weight) for five

Table 2: Experimental design for 15 days chronic toxicity study

A (Naïve Control)	B (Cd Control)	C (Anthocyanin Control)	D (Cd + Anthocyanin Pre-treatment)	E (Cd + Anthocyanin Post-treatment)
Feed and water only	Cadmium (3mg/kg body weight for 5 days)	anthocyanin extract (3g/kg body weight)	Anthocyanin (3g/body weight) for ten consecutive days then Cd (3mg/kg body weight) for the remaining five days	Cd (3mg/kg body weight) for the first five consecutive days then Anthocyanin (3g/kg body weight) for the remaining ten days

Twenty-four hours after the last exposure, the animals were euthanized by cervical dislocation. The abdominal regions were opened exposing the heart, liver and other organs. Blood samples were obtained by cardiac puncture. Blood sample for haematological studies were placed in EDTA containers while those for biochemical assays were placed in heparinized bottles and centrifuged at 3000g for 10 min. From each rat, the kidney and liver, were obtained. They were weighed and 1 g portion of each homogenized in ice-cold saline (1:4, w/v) and centrifuged (Remi motor Ltd) at 5000g for 10 min. Sera and supernatants collected were stored frozen until used for analysis.

Assay kits for AST, ALT, ALP, total protein, urea and creatinine were products of Randox laboratories Ltd, UK. All other reagents used in this study were of analytical grade.

Determination of the activity of aspartate aminotransferase (AST) using Randox Assay Kit

The activity of AST was assayed by the method of Reitman and Frankel (1957), based on the principle that aspartate amino transaminases catalyzes a reaction in which glutamate and pyruvate are produced when α-oxoglutarate receives an amino group from L-aspartate. The activity of AST was thus measured by monitoring the concentration of oxalocetate hydrazone formed with 2,4- dinitrophenylydrazine at a wavelength of 546nm using a spectrophotometer (UV/VIS. Model 752)-

Determination of the activity of alanine aminotransferase (ALT) using Randox Assay Kit

The activity of ALT, like that of AST, was determined using the Reitman and Frankel (1957) approach, which is based on the fact that alanine amino transaminases catalyze a process in which -Oxoglutarate and L-alanine combine to generate glutamate and alanine. Alanine aminotransferase (ALT) activity was determined by measuring the concentration of pyruvate hydrazone produced with 2, 4-dinitrophenyl hydrazine at 546nm.

Determination of the activity of alkaline phosphatase (ALP)

The activity of plasma and tissue ALP were estimatedwas determined by the method of Annino and Giese (1976). The assay is based on the principle that p-nitrophenol is produced from p-nitrophenyl phosphate when acted upon by ALP.

Determination of total protein concentration

Total protein concentration was determined by the biuret method as described by Tietz (1995) following the Randox assay kit protocol. The assay is according to the facts that in an alkaline media, cupric ions react with protein peptide bonds to form a coloured complex with which has highest absorbance at 546 nm and which is proportional to the protein content in the sample.

Estimation urea concentration

This was done according to the principles of Weather burn, (1967) using Randox Assay Kit (Urease-Berthelot Method). Urea in serum was broken down to ammonia in the company of urease. The ammonia was then measured photometrically by Bethelot's reaction.

Determination of serum creatinine

Serum creatinine was determined following the principle of Bartels and Bohmer, (1972) using Randox Assay Kit. Creatinine in alkaline solution reacts with picric acid to form a coloured complex, which is directly proportional to the creatinine concentration.

Statistical analysis

The results are shown as Mean ± SD. The Statistical Package for Social Sciences (SPSS) software was used for statistical analysis (version 21). The Least Significant Difference (LSD) test was used to determine the significance of different parameters using one-way analysis of variance (ANOVA). The difference between means was declared significant at p 0.05 using the Least Significant Difference (ANOVA).

RESULTS AND DISCUSSION

The Effect of *H. sabdariffa* anthocyanin on organ/body weight ratio of Cd-exposed rats. Table 3 illustrates the outcome of administering *H. sabdariffa* anthocyanins on the organ/body weight ratio in rats. The liver and kidney organ/body weight ratios were considerably reduced ($p<0.05$) following acute and subchronic Cd exposure (Group B). In contrast, no significant difference in this parameter was seen in rats given *H. sabdariffa* anthocyanins (anthocyanin control) for acute therapy vs control.

The effect of acute and sub-chronic exposures to Cd on the organ/body weight ratio for the liver and kidney was reversed after pre and post treatments with anthocyanin from *H. sabdariffa* as the values obtained were considerably ($p<0.05$) greater than those of rats exposed to Cd alone. Thus, administration of Cd to experimental animals decreased their organ/body weight ratio for liver and kidney whereas post treatment with anthocyanins from *H. sabdariffa* restored the values towards that of naive control.

Table 3. Effect of *H. sabdariffa* anthocyanins on organ/body weight ratio of Cd-exposed rats

Groups	Organ/body weight ratio (g)	
	Liver	Kidney
5 Days Exposure		
A	4.05 ± 0.19[a]	1.02± 0.21[a]
B	3.23 ± 0.27[b]	0.74 ± 0.27[b]
C	4.03 ± 0.42[a]	0.90 ± 0.24[a]
D	3.85 ± 0.47[c]	0.82 ± 0.29[c]
E	4.00 ±0.56[a]	0.97 ± 0.36[a]
15 Days Exposure		
A	5.68 ± 0.12[a]	1.44 ± 0.11[a]
B	4.33 ± 0.17[b]	1.14 ± 0.17[b]
C	5.43 ± 0.22[a]	1.26 ± 0.22[c]
D	5.46 ± 0.27[a]	1.32 ± 0.19[c]
E	5.37 ± 0.16[a]	1.40 ± 0.14[a]

The values are reported as mean ± standard deviation (SD). n=4. Means in column with altered letters differ substantially ($p<0.05$) within the same tissue and treatment period/time. A (Nave Control), B (Cd Control), C (Anthocyanin Control), D (Anthocyanin Pre-Cd), E (Anthocyanin Post-Cd)

Effect of *H. sabdariffa* anthocyanins on liver function parameters of Cd-exposed rats

The Effect of *H. sabdariffa* anthocyanins on the activities of alanine aminotransferase in the serum and liver of rats administered cadmium is presented in Table 4.

Table 4. Effect of *H. sabdariffa* anthocyanins on the activities of alanine aminotransferase in the serum and liver of rats exposed administered cadmium

Groups	ALT (U/L)	
	Serum	Liver
5 Days Exposure		
A	78.05 ± 3.12[a]	102.24 ± 3.78[a]
B	120.34 ± 2.14[b]	55.56 ± 2.10[b]
C	75.23 ± 1.43[a]	99.87 ± 3.30[a]
D	84.34 ± 2.19[c]	70.38 ± 2.15[c]
E	80.80 ± 3.54[a]	71.38 ± 3.48[c]
15 Days Exposure		
A	72.23 ± 2.32[a]	134.40 ± 3.40[a]
B	125.02 ± 2.23[b]	48.64 ± 2.80[b]
C	70.90 ± 2.33[a]	132.04 ± 3.20[a]
D	71.24 ± 2.96[a]	64.28 ± 2.18[c]
E	71.10 ± 3.04[a]	65.24 ± 3.62[c]

The values are reported as mean ± standard deviation (SD). n=4. Means in column with altered letters differ substantially ($p<0.05$) within the same tissue and treatment period/time. A (Nave Control), B (Cd Control), C (Anthocyanin Control), D (Anthocyanin Pre-Cd), E (Anthocyanin Post-Cd)

There was no statistically significant change in serum ALT activity of rats in the naïve control and those given HS anthocyanin (Group C), but exposure to Cd alone (Group B) significantly increased ($p<0.05$) ALT activity. This was observed for both treatment periods. Also, for the two periods, pre-treatment and post treatment of Cd-exposed rats with HS anthocyanins significantly decreased ($p<0.05$) the elevation in ALT activity caused by Cd exposure. However, rats given HS anthocyanins after Cd exposure (Group E) had lower ALT activity than those pre-treated with HS anthocyanins before Cd exposure (Group D) for the 5 days acute exposure period.

ALT activity in the liver followed a slightly different trend. Exposure to Cd alone (Group B) significantly lowered ($p<0.05$) ALT activity in the liver for both treatment periods compared to the naïve control. However, as detected in the serum, pre-treatment and post-treatment of Cd-exposed rats with HS Anthocyanins significantly increased ($p<0.05$) the observed reduction in ALT activity caused by Cd exposure.

From the results, it can be deduced that acute and chronic exposure to Cd significantly increased ALT activity in the serum with a corresponding decrease in the liver, but the Cd-induced changes were improved towards normal by treatment of Cd-exposed rats with HSA before and after Cd exposure.

Table 5 displays the effect of administration of *H. sabdariffa* anthocyanins on the activities of Aspartate Aminotransferase in the serum and liver of rats exposed to cadmium

Table 5. Effect of H. sabdariffa anthocyanins on the activities of aspartate aminotransferase in the serum and liver of rats exposed to cadmium

Groups	AST (U/L)	
	Serum	Liver
5 Days Exposure		
A	120.22 ± 4.18[a]	196.34 ± 3.14[a]
B	204.25 ± 3.12[b]	112.30 ± 3.14[b]
C	122.46 ± 2.94[a]	189.46 ± 3.15[a]
D	138.24 ± 3.15[c]	171.36 ± 2.26[c]
E	130.28 ± 3.26[c]	178.80 ± 3.16[c]
15 Days Exposure		
A	127.12 ± 3.28[a]	233.34 ± 3.10[a]
B	208.20 ± 3.23[b]	101.38 ± 3.04[b]
C	132.60 ± 1.68[a]	220.01 ± 3.05[c]
D	160.44 ± 3.15[c]	205.45 ± 2.62[d]
E	152.84 ± 3.26[c]	215.05 ± 3.14[e]

The values are reported as mean ± standard deviation (SD). n=4. Means in column with altered letters differ substantially ($p<0.05$) within the same tissue and treatment period/time. A (Nave Control), B (Cd Control), C (Anthocyanin Control), D (Anthocyanin Pre-Cd), E (Anthocyanin Post-Cd)

In the serum, the action of AST was considerably increased ($p<0.05$) in rats exposed to Cd alone (Group B) relative to the naïve control (Group A). Conversely, a major decrease ($p<0.05$) was recorded in AST activity in the liver of rats exposed to Cd alone (Group B) compared with to the naïve control. This was seen in both experimental periods.

AST activity in rats given HSA was not significantly different from the control in the liver (with the exception of the liver for the sub-chronic exposure). Here, treatment of rats with HSA, significantly decreased($p<0.05$) AST activity relative to the naïve control, but significantly higher ($p<0.05$) than the Cd alone group (Group B).

For both modes of exposure and in the serum and liver, treatment of Cd-exposed rats with HSA before and after Cd exposure (Groups D and E) significantly altered AST activity relative to rats administered Cd alone (Group B). In the serum, a significant decrease($p<0.05$) in AST activity was observed while in the liver, a significant increase in AST activity was seen in rats Cd-exposed rats pre and post treated with HSA compared to rats administered Cd alone.

The results indicate that administration of Cd to rats in both modes of exposure significantly increased ($p<0.05$) AST activity in the serum with a corresponding decrease in the liver, but these changes were improved towards normal by pre and post treatment of Cd-exposed rats with HSA.

The Effects of administration of *H. sabdariffa* anthocyanins on the activities of Alkaline Phosphatase in the serum and liver of rats exposed to cadmium is shown in Table 6.

Table 6. Effect of *H. sabdariffa* anthocyanin on the activities of Alkaline Phosphatase in the serum and liver of rats exposed to cadmium

Groups	ALP (U/L)	
	Serum	Liver
5 Days Exposure		
A	144.83 ± 3.88[a]	210.04 ± 3.24[a]
B	196.16 ± 3.28[b]	101.98 ± 3.26[b]
C	152.16 ± 2.98[a]	201.98 ± 2.26[a]
D	159.24 ± 2.09[a]	190.29 ± 2.46[c]
E	158.50 ± 2.50[a]	192.45 ± 3.56[c]
15 Days Exposure		
A	166.91 ± 3.10[a]	211.44 ± 1.64[a]
B	204.44 ± 3.28[b]	115.88 ± 2.44[b]
C	165.78 ± 2.62[a]	210.24 ± 2.64[a]
D	170.20 ± 2.78[a]	199.24 ± 2.50[a]
E	169.68 ± 3.60[a]	199.88 ± 2.68[a]

The values are reported as mean ± standard deviation (SD). n=4. Means in column with altered letters differ substantially ($p<0.05$) within the same tissue and treatment period/time. A (Nave Control), B (Cd Control), C (Anthocyanin Control), D (Anthocyanin Pre-Cd), E (Anthocyanin Post-Cd)

Contact to Cd alone (Group B) significantly increased ($p<0.05$) the activity of ALP in the serum for both modes of exposure compared to the naïve control. There was no significant difference ($p>0.05$) in ALP activity in rats treated with HSA compared to naïve control. In addition, pre and post treatment of Cd-exposed rats with HSA significantly reduced ($p<0.05$) serum ALP activity compared to rats exposed to Cd alone (Group B) bringing the activity of the enzyme to a level similar with and comparable to the naïve control. This effect was seen in both the acute and sub-chronic exposures.

In the liver, the activity of the enzyme was significantly reduced ($p<0.05$) in rats exposed to Cd alone (Group B) compared to the naïve control for both the acute and sub-chronic exposures. Also, for the two modes of exposure, treatment of rats with HSA did not significantly ($p>0.05$) alter the activity of ALP in the liver relative to the naïve control. However, when Cd-exposed rats were pre and post treated with HSA, a significant increase ($p<0.05$) in the activity of the enzyme was observed in the liver compared with rats exposed to Cd alone (Group B).

The inference from this result is that exposure to Cd significantly altered ALP activity in the serum and liver of rats relative to control. However, the administration of HSA to Cd-exposed rats was able to restore ALP activity to a level comparable with the naïve control.

Table 7: Effect of *H. sabdariffa* anthocyanins on Protein level in the plasma and liver of rats exposed to cadmium

Groups	Protein (mg/ml)	
	Serum	Liver
5 Days Exposure		
A	41.50 ± 1.28[a]	6.44 ± 0.22[a]
B	50.34 ± 1.16[b]	7.36 ± 0.12[b]
C	40.56 ± 2.14[a]	6.35 ± 0.13[a]
D	43.56 ± 1.99[c]	7.10 ± 0.32[c]
E	42.60±2.15[a]	7.05 ± 0.29[c]
15 Days Exposure		
A	43.23 ± 2.38[a]	4.95 ± 0.15[a]
B	70.40 ± 2.20[b]	6.80 ± 0.23[b]
C	43.58 ± 2.25[a]	4.78 ± 0.11[a]
D	44.67 ± 2.09[a]	5.98 ± 0.14[c]
E	43.68 ± 2.85[a]	6.00 ± 0.18[c]

The values are reported as mean ± standard deviation (SD). n=4. Means in column with altered letters differ substantially ($p<0.05$) within the same tissue and treatment period/time. A (Nave Control), B (Cd Control), C (Anthocyanin Control), D (Anthocyanin Pre-Cd), E (Anthocyanin Post-Cd)

The effects of administration of H. sabdariffa anthocyanins on protein level in the plasma and liver of rats exposed to cadmium is presented in Table 7. In both treatment periods, exposure to Cd alone (Group B) significantly increased ($p<0.05$) serum protein compared to the naïve control while there was no significant difference ($p>0.05$) between serum protein levels of control animals and those maintained on HS anthocyanins without exposure to Cd. However, pre-treatment and post-treatment of Cd-exposed rats with HS anthocyanins significantly reduced ($p<0.05$) elevated serum protein levels caused by Cd exposure.

The 15 days treatment reduced serum protein levels elevated by pre and post administration of HS anthocyanins to Cd-exposed rats to a level not significantly different from that of the naïve control animals, whereas in the 5 days treatment, this was only observed in Group E.A similar trend was observed for liver protein; however, exposing rats treated with Cd to HS anthocyanins before and after Cd exposure significantly reduced liver protein compared to rats maintained on Cd alone although this reduction was significantly higher ($p<0.05$) than the naïve control group.

It can thus be inferred that exposure to Cd significantly increased ($p<0.05$) protein levels in the serum and liver of Cd-exposed rats compared to naïve control, and the administration of HSA improved protein levels towards normal.

Effect of *H. sabdariffa* anthocyanin on kidney function parameters of Cd-exposed rats

The effect of administration of *H. sabdariff* aanthocyanin on the levels of creatinine and Urea in the serum of rats exposed to Cd is shown in Table 8. Exposure to Cd alone (Group B) significantly increased serum creatinine and urea compared to the naïve control, pre-treatment and post-treatment of Cd-exposed rats with HSA significantly reduced serum creatinine and urea to a level significantly different from rats maintained on Cd alone but not significantly different from the control.

Table 8. Effect of *H. sabdariffa* anthocyanin on the levels of creatinine and Urea in the serum of rats exposed to Cd

Groups	Parameters	
	Creatinine (mg/dl)	Urea (mg/dl)
5 Days Exposure		
A	0.52 ± 0.03[a]	21.68 ± 1.05[a]
B	0.89 ± 0.11[b]	28.22 ± 2.12[b]
C	1.64 ± 0.10[c]	23.54 ± 1.25[a]
D	0.51 ± 0.05[a]	20.15 ± 2.10[a]
E	0.52 ± 0.07[a]	19.98 ± 1.09[a]
15 Days Exposure		
A	1.75 ± 0.11[a]	23.86 ± 1.16[a]
B	2.09 ± 0.15[b]	30.28 ± 2.28[b]
C	1.64 ± 0.10[c]	23.54 ± 1.25[a]
D	1.90 ± 0.11[d]	22.95 ± 2.12[a]
E	1.89 ± 0.08[d]	25.66 ± 1.94[c]

The values are reported as mean ± standard deviation (SD). n=4. Means in column with altered letters differ substantially ($p<0.05$) within the same tissue and treatment period/time. A (Nave Control), B (Cd Control), C (Anthocyanin Control), D (Anthocyanin Pre-Cd), E (Anthocyanin Post-Cd)

Discussion

This study investigated the effects of administration of *Hibiscus sabdarrifa* L. anthocyanins on cadmium induced hepato-nephro toxicity in rats.

The fact that anthocyanin pigments are potent medicinal agents has been long accepted in folk medicine throughout the world (Rechner and Kroner, 2005). These pigments are linked to a great variety of health benefits such as lowering the risk of cardiovascular disease, diabetes, arthritis and cancer (Wang and Stoner, 2008, Jaganath and Crozier, 2010).Thus, Increase in organ/body weight gain caused by administration *H. sabdarrifa* extracts following pre and post treatment may be attributed to the nutritional components therein, its antioxidant properties and possible effects on the digestion and absorption of nutrients (Asagba *et al*., 2007; Dahiru *et al*., 2013). Changes in organ to body weight ratio may reveal swelling in organs, organ deterioration or hypertrophy, indicating the constriction and/or inflammation

of cells (Moore and Dalley, (1999, Amresh *et al.*, 2008). Thus, the significant reduction in kidney/body weight observed in rats maintained on Cd alone (Group 2) compared to control is a sign of Cd-induced damage to the normal functioning of organs and agrees with the work of De Souza Predes *et al*. (2010). On the other hand, the fact that the administration of HSA did not cause any significant change in organs-body weight ratio compared to naïve control, is an indication that the extract did not induce swelling or atrophy of the hepatocytes and nephrocytes, while increase in the organs/body weight ratio observed when Cd-exposed rats were pre-treated and post-treated with HSA can be attribute to the protective and curative effects of HSA against Cd-induced changes in the organs/body weight ratio. Asagba*et al*. (2007) and Hashemi (2014) had previously reported increase in body weight gain and organ/body weight ratio by administration of HS aqueous extract relative to Cd-exposed rats. The results are however contrary to the work of Alzubade, (2014) where the administration of aqueous extract of *H. sabdariffa* (at dosage of 25, 50, 100 and 200 mg/kg body weight) did not significantly ($p>0.05$) change liver and kidney/body weight ratio compared with control. This variance may be attributed to differences in feed, treatment methods, exposure periods, dose of extract as well as the presence of other constituents in their extract. According to Asagba *et al*. (2007), and Dahiru *et al.* (2013), the protection of examined organs by the extract indicated by enhanced organ/body weight gain, suggests that *H. sabdarrifa* L. Anthocyanins might be curative and protective in Cd toxicity as it significantly neutralized the toxic effects of Cd and helped in the regeneration of hepatocytes and the cells of the kidneys due to its antioxidant activity.

As shown in Tables 2 and 3, there was a significant increase in the activities of ALT and AST in the serum with a decrease in the liver following Cd exposure. This is an indication that the Cd exposure resulted in cytotoxic injury in the liver. According to Yakubu *et al*. (2003) and Nafiu et al. (2011), determining the degree of attack and toxicity of a chemical molecule on organs/tissues may be done by measuring the activity of "marker" enzymes or biomarkers in tissues and bodily fluids. This result agrees with reported toxic effects of Cd in the liver of rats (Orororo *et al*., 2017; Imafidon, 2018; Ndubuisi *et al*., 2020). Cd-exposure also caused a significant elevation in the activities of Alkaline phosphatase (ALP) in the serum with a corresponding decrease in the liver (Table 4). Alkaline phosphatase (ALP), found predominantly in the plasma membrane and endoplasmic recticulum (Shahjahan *et al*., 2004), is generally used alongside aminotransferases (AST and ALT) to assess plasma membrane integrity and normal function of the liver as increase in the activity of these enzymes (especially in the plasma) is indicative of possible damage to the membrane of cells (Akanji *et al*., 1993; Yakubu *et al.*, 2007). The increased activity of these enzymes in the plasma may be as a result of their leakage into blood stream due to the Cd-induced compromise of liver membrane integrity. On the other hand, a significant decrease in the activities of these enzymes indicates a decrease in damage to cells and tissues. Thus, the reduction in ALP, AST, and ALT activities observed in the plasma of Cd-exposed rats pre- and post-treatment with HS anthocyanins implies a decrease in the incidence of tissue cell injury and is consistent with the extract's observed protective effect against cadmium-induced membrane lipid peroxidation (Orororo *et al.,* 2018). In other words, pre- and post-treatment with HS anthocyanins reduced Cd-induced hepatocytotoxic damage, as measured by the activity of the biomarkers aminotransferases and alkaline phosphatase, most likely through counteracting Cd-induced oxidative stress and membrane lipid peroxidation (Orororo *et al*., 2018). Total protein is a measure of all plasma proteins in the blood that can be modified by changes in protein synthesis in the liver, protein distribution, and protein breakdown and excretion (Kolawole *et al*., 2011). According to Kolawole *et al.* (2014), a rise in total protein is typically caused by tissue injury. In this investigation, administration of HS anthocyanins generated no significant changes in total protein levels, showing that the extract did not cause any substantial harm to the liver or modify protein clearance from the body. According to Kolawole *et al* (2014), a rise in total protein is typically caused by tissue injury. In this investigation, administration of HS anthocyanins generated no significant changes in total protein levels, showing that the extract did not cause any substantial harm to the liver or modify protein clearance from the body.

The effect of *H. sabdariffa* anthocyanin on serum creatinine and urea levels shown in Table 8 suggests that renal function was not compromised following administration of the extract, which is in line with the discovery of Abbas et al. (2011), who concluded that *H. sabdariffa* was a highly beneficial and safe medicinal plant because there was no major harmful change in serum creatinine and urea levels after administration to rats. Furthermore, blood creatinine levels in Cd-exposed rats pre- and post-treated with HSA were considerably ($p<0.05$) lower than in rats open to Cd alone (Group C), indicating HSA's nephroprotective efficacy.

This study confirms the ability of HSA to protect the liver and kidney against Cd toxicity. That the administration of HS anthocyanins did not alter the activities of ALT, AST,

ALP, urea and creatinine compared to the control is further evidence of the safety at the dose studied.

CONCLUSION

The outcome of this study showed that cadmium is toxic and altered the activities of the liver and kidney function indices. However, pre-treatment and post-treatment of Cd-exposed rats with *H. sabdariffa* anthocyanins significantly reversed the toxic effects of Cd-exposure. The observed effects were both curative and protective. This indicates that HS anthocyanins are potent in ameliorating Cd-induced hepato-nephro-toxicity in male Wistar rats.

AUTHORS' CONTRIBUTIONS

OCO and SOA designed the experiment, carried out the laboratory work, wrote and edited the article. Both authors read and approved the final version for publication.

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None

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

ETHICAL APPROVAL

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed (Olfert *et al*., 1993)

REFERENCES

Abbas, M., Shirin, M., Patricia, K. and Mohammad, G. K. (2011). The effect of *Hibiscus sabdariffa* on lipid profile creatinine, and serum electrolytes: a randomized clinical trial. *Gastroenterology*, 4:23-27.

Ajiboye, T.O., Salawu, N.A., Yakubu, M.T., Oladiji, A.T., Akanji, M.A. and Okogun, J.I. (2011). Antioxidant and drug detoxification potentials of *Hibiscus sabdariffa* anthocyanin extract. *Drug and Chemical Toxicology*, 34(2): 109–115.

Akanji, M. A., Adegoke, O.A. and Oloyede, O.B. (1993). Effect of chronic consumption of metabisulphate on the integrity of rat cellular system, *Toxicology*, 81: 173–179.

Alzubade, B. (2014). Effects of aqueous extract of *Hibiscus sabdariffa* L. on some biochemical indices of liver and kidney function in male albino rats. *Magazin o Al-Kufa University Biology*, 6 (2): 1-9.

Amresh, G.R, Singh, P.N. and Rao, V.C. (2008) Toxicological screening of traditional medicine Laghupatha (Cissampelospareira) in experimental animals. *Journal of Ethnopharmacology*, 116:454–460.

Annino, J.S. and Giese R. W (1976) Clinical chemistry.principles and procedure, 4th edn. Boston: Little Brown.

Asagba, S.O. (2010). Biochemical changes in urine and plasma of rats in food-chain mediated cadmium toxicity. *Nigerian Journal of Biochemistry and Molecular Biology*, 25 (1): 9 – 17.

Asagba, S.O., Adaikpoh, M.A., Kadiri, H. and Obi, F.O. (2007). Influence of aqueous extract of *Hibiscus sabdariffa* L. petal on cadmium toxicity in rats. *Biological Trace Element Research*, 115(1): 47-57.

Ayakeme, G. T., Ibeh, O., Nwinuka, M. N. and Nwachoko, N. C. (2012). Effects of garlic extract on cadmium-induced toxicity in Wistar albino rat. *Indian Journal of Drugs and Diseases*, 1(3): 68-72.

Bartels, H. and Bohmer, M. (1972). Micro-determination of creatinine. *Clinical Chimica.Acta*, 32:81-85.

Beutler, E., Duron, O. and Kelly, B.M. (1963). Improved method for the determination of blood glutathione. *Journal of Laboratory Clinical Medicine*, 61:882-888.

Chakraborty, S., Dutta, A.R., Sural, S., Gupta, D. and Sen, S. (2013). Ailing bones and failing kidneys: a case of chronic cadmium toxicity. *Annals of Clinical Biochemistry*, 50(5) 492–495.

Chalker-Scott, L. (1999). Environmental significance of anthocyanins in plant stress responses. *Photochemistry and Photobiology*, 70:1-9.

Dahiru, D., Umaru, H.A, Vasira, B., Agama, A.M. (2013).Antioxidant and antiperoxidative activities of aqueous extract of *Hibiscus sabdariffa* L. calyx on alcohol-induced hepatic lipid peroxidation in rats. *American Journal of Biochemistry*, 3(2): 62-65.

De Souza Predes, F., Diamante, M.A. and Dolder, H. (2010). Testis response to low doses of cadmium in Wistar rats. *International Journal of Experimental Pathology*, 91:125-131.

Elias, A., Oputiri, D. and Oru-Bo, P.G. (2013). Hepatotoxicity of cadmium and roles of mitigating agents. *British Journal of Pharmacology and Toxicology*, 4(6): 222-231.

El-Sokkary, G. H. and Awadalla, E. A. (2011). The protective role of vitamin C against cerebral and pulmonary damage induced by cadmium chloride in male adult albino rat. Open *Neuroendocrinology Journal*, 4, 1-8.

Hashemi, J.M. (2014). *Hibiscus sabdariffa* calyx extract alleviate hepatotoxicity induced by carbon tetrachloride on male albino rats. *Nature and Science*, 12(6):111-120.

Hayati, A., Pratiwi, H., Khoiriyah, I., Winarni, D., Sugiharto, E. (2017). Histopathological assessment of cadmium effect on testicles and kidney of Oreochromisniloticus in different salinity. Citation: AIP Conference Proceedings 1854, 020014;

Imafidon, C.E., Akomolafe, R.O., Abubakar, S.A., Ogundipe, O.J., Olukiran, O.S., Ayowole, O.A. (2015). Amelioration of cadmium-induced nephropathy using polyphenol-rich extract of *Vernonia amygdalina* (del.) leaves in rat model. *OA Macedonia Journal of Medical Sciences*, 3(4):567-577.

Imafidon, C. E., Olukirana, O. S., Ogundipea, D. J., Eluwoleb, A. O., Adekunlea, I. A. and Oke, G. O. (2018). Acetonic extract of *Vernonia amygdalina* (Del.) attenuates Cd-induced liver injury: potential application

in adjuvant heavy metal therapy. *Toxicology Reports* 5: 324–332.

Jaganath, I. B. and Crozier, A. (2010). Dietary flavonoids and phenolic compounds. In *Plant Phenolics and Human Health*: Biochemistry, Nutrition, and Pharmacology (edited by Cesar G. Fraga). John Wiley & Sons, Inc., Hoboken, New Jersey.pp. 23.

Kamal, S. (2010). Nephroprotection of lacidipine against gentamycin-induced nephrotoxicity in albino rats. *Journal of Experimental Biology*, (2): 59-63.

Kolawole, O. T., Akiibinu, M. O. and Akanji, M. A. (2014) Assessment of the effect of aqueous extract of calyx of *Hibiscus sabdariffa* on some biochemical indices of renal function in rats. *International Journal of Pharmaceutical Sciences*, 4 (3): 587-590.

Kolawole, S. O., Kolawole, O. T., Akanji, M. A. (2011). Effects of aqueous extract of Khayasene galensis stem bark on biochemical and hematological parameters in rats. *Journal of Pharmacology and Toxicology*, 6 (6): 602-607.

Liebelt, E. L. and Shannon, M.W. (1994). Oral chelators for childhood lead poisoning. *Pediatrics Annals*, 23: 616–626.

Mazza, G. J. (2007) Anthocyanins and heart health. *Annals of Istanbul Super Sanità*; 43: 369-374.

McCarty, M. F. (2012). Zinc and multi-mineral supplementation should mitigate the pathogenic impact of cadmium exposure. *Medical Hypotheses*, 79: 642–648.

Messner, D.J., Sivam, G., Kowdley, K.V. (2010). Curcumin reduces the toxic effects of iron loading in rat liver epithelial cells. *Liver International*, 29: 63–72.

Monachese, M., Burton, J.P. and Reid, G. (2012). Bioremediation and tolerance of humans to heavy metals through microbial processes: A potential role for probiotics? *Applied Environmental Microbiology*, 78: 6397–6404.

Moore, K. L. and Dalley, A.F. (1999). Structure of the penis. In: *Clinical Oriented Anatomy*. 4th edition. Lippincot Williams and Williams; a WollerKlumner Corporation, Philadelphia: 287-299.

Mortn, J. (2010). Roselle. Fruits of warm climates. P. 281-286. Miami, FL

Nafiu, M.O., Akanji, M. A. and Yakubu, M. T. (2011) Effect of aqueous extract of *Cochlospermum planchonii* rhizome on some kidney and liver functional indicies of albino rats. *African Journal of Traditional and Complementary Alternative Medicine*, 8(1): 22–26.

Ndubuisi, A., Ani, C., Eze W., Ugwudike, P., Anyaeji, P., Ude, V. C., Agu, F. U. Nworgu, C. and Ikwuka, D. (2020). The effect of aqueous extract of zest of citrus sinensis(AEZCs) on cadmium chloride induced liver toxicity in Wistar rats. *African Journal of Biochemistry Research*, 14(1):5-17.

Nordberg, G.F., Nogawa, K. and Nordberg, M. (2011). Cadmium. In: Nordberg GF, Fowler B, Nordberg M, Friberg LT. eds. *Handbook on the toxicology of metals*. 3rd edn. Academic Press, Elsevier, 2007:445–86.

Olfert, E. D., Cross, B. M., McWilliams, A. A. (1993). *Guide to the care and use of experimental animals*. 2nd ed. Ottawa, Canada: Canadian Council on Animal Care; p. 82-93.

Orororo, O. C., Asagba, S. O., Oghri, E. and Egbune, E. E. (2017). Comparative effect of garden egg, carrot and oat on biochemical parameters in cadmium exposed rats. *African Journal of Biochemistry Research*, 12(3): 28-34.

Orororo, O. C., Asagba, S. O, Tonukari, N. J., Okandeji, O. J. and Mbanugo, J. J. (2018). Effects of *Hibiscus sabdarrifal*.anthocyanins on cadmium-induced oxidative stress in wistar rats. *Journal of Applied Science and Environmental Management*, 22 (4): 465-470.

Prozialeck, W.C., Edwards, J.R. (2012). Mechanisms of cadmium-induced proximal tubule injury: new insights with implications for biomonitoring and therapeutic interventions. *Journal of Pharmacology and Experimental Therapeutics*, 343: 2-12.

Rajasekaran, A. and Periasamy, M. (2012). Hepatoprotective effect of ethanolic leaf extract of *Calycopteris floribunda* Lam on cadmium induced hepatotoxicity in rats. *Research Journal of Pharmaceutical Biology and Chemical Sciences*, 3 (3): 381-389.

Rechner, A. R. and Kroner, C. (2005). Anthocyanins and colonic metabolites of dietary polyphenols inhibit platelet function. *Thrombosis Research*, 116: 327-334.

Reitman, S. and Frankel, S. (1957). Glutamic – pyruvate transaminase assay by colorimetric method. *American Journal of Clinical Pathology*, 28: 56-63.

Sakr, S.A., Bayomy, M.F. and El-Morsy, A.M. (2015). Rosemary extract ameliorates cadmium-induced histological changes and oxidative damage in the liver of albino rat. *Journal of Basic and Applied Zoology*, 71: 1–9.

Satarug, S., Vesey, D.A., Gobe, G.C. (2017). Kidney cadmium toxicity, diabetes and high blood pressure: the perfect storm. *Tohoku Journal of Experimental Medicine*, 241: 65-87.

Shahjahan, M., Sabitha, K.E., Malbika, J. and Shyamala-Devis, C. S. (2004). Effect of Solanum trilobatum against carbon tetrachloride induced hepatic damage in albino rats. *Indian Journal of Medical Research*, 120: 194–198.

Shaikh, Z. A., Jordan, S. A. and Tang, W. F. (1999) Protection against chronic cadmium toxicity by caloric restriction. *Toxicology*, 133: 93–103.

Song, J.K.,Luo, H., Yin, X.H., Huang, G.L., Luo, S.Y., Lin, D.R., Yuan, D.B., Zhang, W.andZhu, J.G. (2015). Association between cadmium exposure and renal cancer risk: a meta-analysis of observational studies. *Scientific Reports*. 5: 17976.

Tietz, N. W. (1995). Clinical guide to Laboratory Tests.3rd Edition. WB Saunders Company, Philadephia. PA pp 518-519.

Vesey, D.A. (2010) Transport pathways for cadmium in the intestine and kidney proximal tubule: focus on the interaction with essential metals. *Toxicological Letters*, 198: 13–19.

Wallin, M., Sallsten, G., Lundh, T., Barregard, L. (2014). Low level cadmium exposure and effects on kidney function. *Occupational Environmental Medicine*, 71: 848-854.

Wang, H. K. (2000) The therapeutic potential of flavonoids. Expert Opinion on Investigation of Drugs, 9: 2103–19.

Wang, L. and Stoner, G.D. (2008). Anthocyanins and their role in cancer prevention. *Cancer Letters*, 269 (2): 281–290.

Weatherburn, M.W. (1967). Urea concentration estimation in samples. *Annals of Chemistry*, 39: 971.

World Health Organization (2011). Evaluation of certain food additives and contaminants. Seventy-third report of the Joint FAO/WHO Expert Committee on Food Additives. Geneva.

Yakubu, M. T., Bilbis, L.S., Lawal, M. and. Akanji, M.A. (2003) Effect of repeated administration of sildenafl citrate on selected enzyme activities of liver and kidney of male albino rats. *Nigerian Journal of Pure and Applied Sciences*, 18: 1395–1400.

Yakubu, M. T., Oladiji, A. T. and Akanji, M. A. (2007). Evaluation of biochemical indices of male rat reproductive function and testicular histology in wistar rats following chronic administration of aqueous extract of *Fadogiaagrestis* (Schweinf. Ex Heirn) stem. *African Journal of Biochemistry Research*, 1 (7):156-163.

Zhai, Q., Narbad, A. and Chen, W. (2015). Dietary Strategies for the treatment of cadmium and lead toxicity. *Nutrients*, 7: 552-571.

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