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Assessing the effect of cadmium and *Hibiscus sabdariffa* calyx extract on the organ gravimetry and lipid profile of the liver and serum of African catfish (*Clarias gariepinus*)

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Abstract

Toxicity of cadmium to all lives especially the aquatic life cannot be quantified due to their deleterious effect usually caused as a result of their incessant and uncontrollable discharged into the aquatic environment. The present study was undertaken to assess the effect of cadmium and *Hibiscus Sabdariffa* Calyx Extract on the Organ Gravimetry and Lipid Profile of the Liver and Serum of African Catfish (*Clarias gariepinus*). Forty juvenile catfish were divided into four groups containing ten fish with average weight 170 ± 2 kg per group [Group A, Control (–HSCE – Cd), Group B (+HSCE), Group C (+Cd) and Group D (+HSCE + Cd)]. Group B and D were administered a daily dose of 40ml/kg body weight of *Hibiscus sabdariffa* calyx extracts (HSCE) (0.25%v/v) for 14 days while groups C and D were exposed to the same dose containing 0.3 mg of Cd/L daily for 14 days. The result shows that there was significant decrease ($p < 0.05$) in the liver total- and LDL-cholesterol of fish exposed to HSCE and HSCE + Cd treated groups when compared to control and cadmium treated group. The triglycerides concentration of liver and serum was significantly increased ($p < 0.05$) in fish exposed to cadmium when compared to the control and HSCE + Cd treated group respectively. SOD activity was significantly increase ($p < 0.05$) in the serum of catfish exposed to Cd when compared to control. However, the liver-body weight ratio was significantly decreased in Cd and Cd + HSCE treated groups relative to the control. It is evident from the result obtained in this study that HSCE has protective effect against cadmium intoxicated fish (*Clarias gariepinus*).

1. Introduction

One of the primary sites for the contamination of river water with heavy metals is the waste from nearby industries. The contamination of fresh waters with a wide range of pollutants has become a matter of concern over the last few decades (Vutukuru, 2005). The natural aquatic systems may extensively be contaminated with heavy metals released from domestic, industrial and other man-made activities (Velez and Montoro, 1998). Heavy metal contamination is now known to have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms (Farombi *et al.*, 2007).

In fish, cadmium can cause a number of structural and pathomorphological changes in various organs. The highest cadmium levels were detected in the kidneys and liver of fish (Thophon *et al.*, 2003). Cadmium is noted for its tendency to accumulate in the organisms of mammals for a prolonged biological semi-life. It is responsible for increased hypertension, emphysema, kidney tubule damage, impaired liver function, and cancer (Ribelin and Migaki, 1975).

Studies have shown that, in the case of acute cadmium poisoning, the primary site of toxicity are the gills' lamellae and kidney tubules. In the case of sub-chronic poisoning, the primarily affected organs appear to be kidneys and liver, and to a lesser extent the gills (Thophon *et al.*, 2003). Among animal species, fishes are the inhabitants that cannot escape from the detrimental effects of these pollutants (Olaifa *et al.*, 2004) and as such fish are widely used to evaluate the health of aquatic ecosystems because pollutants build up in the food chain and are responsible for adverse effects and death in the aquatic systems (Farkas *et al.*, 2002).

Studies carried out on various fishes have shown that heavy metals may alter the physiological activities and biochemical parameters both in tissues and in blood (Kalay and Canli, 2000). The toxic effects of heavy metals have been reviewed, including bioaccumulation (Waqar, 2006). The organisms developed a protective defense against the deleterious effects of essential and nonessential heavy metals and other xenobiotic that produce degenerative changes like oxidative stress in the body (Abou EL-Naga *et al.*, 2005). A variety of contaminants

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including toxic heavy metals (cadmium, copper, mercury and zinc) are reported to be ubiquitously present in rivers, reservoirs and are disadvantageous for aquatic organisms (Olsson, 1998).

However, they are not biodegraded and therefore, their bioaccumulation in fish, oyster, mussels, sediments and other components of aquatic ecosystems have been reported from all over the world. It appears that problem of heavy metals accumulation in aquatic organisms including fish needs continuous monitoring and surveillance owing to biomagnifying potential of toxic metals in human food chain (Kumar et al., 2009).

Recent reports show that *Hibiscus sabdariffa* calyx aqueous extract offers protection against cadmium-induced decrease in total- and LDL- cholesterol of African catfish (Obi et al., 2014). Also red calyces of *Hibiscus sabdariffa* extract has been shown to contain potent antioxidant principles (Ologundudu and Obi, 2005; Ologundudu et al., 2009a; Ologundudu et al., 2010). In fish, like other vertebrates the liver is the key tissue involve in the detoxification of xenobiotic, thus it is a useful organ for the evaluation of any changes in the quality of water, as it is sensitive to any detrimental changes (Patel and Bahadur, 2011). The aims of the present study were therefore to assess the effects of cadmium as a possible stress agent and to ascertain whether the indices produced, if any, will be minimized in the presence of aqueous extract of *Hibiscus sabdariffa* calyces in the cadmium contaminated water.

2. Material and methods

2.1 Materials

Fish

Forty juvenile African Catfish (*Clarias gariepinus*) of average weight 170 ± 2 kg per group used for this study were obtained from a commercial fish farm, Elezebata Fish Farm, Ugbowo, Benin City, Edo State and were acclimatized in the Animal House, Department of Biochemistry, University of Benin for two weeks before the commencement of the study. To use animals for this research, The Research Ethical Committee of the Biochemistry Department, University of Benin was contacted. Handling of animals was carried out in accordance with the recommended international standard.

Plant Materials

The plant material, *Hibiscus sabdariffa* Linn calyces were purchased from Uselu Market, Ugbowo, Benin City, Edo State, Nigeria.

Reagents and Chemicals

Analytical grade sodium chloride, cadmium chloride monohydrate ($\text{CdCl}_2 \cdot \text{H}_2\text{O}$), glacial acetic acid, sodium hydrogen carbonate, EDTA, sodium hydroxide, used for this study were the product of British Drug House (BDH) Poole, England, epinephrine, hydrochloric acid, potassium tetraoxomanganate (vii), disodium hydrogen phosphate, potassium dihydrogen phosphate (May and Baker, Dagenham, England), trichloroacetic acid, 2-thiobarbituric acid (KEM Light Lab. Ltd), anhydrous sodium carbonate (Sigma-Aldrich Inc, St. Louis, USA), tetraoxosulphate (vi) acid (Pyrex, England), hydrogen peroxide (Fharma Trends, Nigeria) while total cholesterol, HDL-cholesterol and triglyceride assay kits were products of Randox, United Kingdom.

2.2 Methods

Preparation of Stock Cadmium Solution and Cadmium Contaminated Water

Stock solution of cadmium chloride was prepared by dissolving 1 g of cadmium chloride monohydrate ($\text{CdCl}_2 \cdot \text{H}_2\text{O}$) in 100 ml of distilled water. Fifty-four microliters of the solution contain the equivalent amount of 0.3 mg Cd (Molecular weight of $\text{CdCl}_2 \cdot \text{H}_2\text{O}$ = 201.4). One thousand three hundred and fifty microlitres of cadmium solution (0.3 mg Cd/L) was introduced into 25 litres of water which was used for the cadmium exposed fish while 25 litres of cadmium-free water was used for the control group.

Preparation of Aqueous Extract of Hibiscus sabdariffa

Hibiscus sabdariffa (160 g) was weighed into a beaker containing 800 ml of distilled water and left standing for 48 hours. At the end of 48 hours, aqueous extract, the red pigment was separated by filtration. The amount of solid residue in 1 ml of the extract after evaporation to dryness in a watch glass was determined as described previously (Obi and Fadairo, 2013; Obi and Uneh, 2003).

Treatment of Fish

Four experimental groups containing ten African cat fish (*Clarias gariepinus*) each were used for this study. The experiment which lasted for two weeks was preceded by two weeks of acclimatization (Ogunbiyi, 2017). The fish were fed on 2 mm vital fish feed (3% of mean body weight) obtained from Animal Farm Ltd. Ikare Akoko, Ondo State. The actual feed was divided into two portions and given twice daily. The water was changed every other day to meet the oxygen demand of the fish and to get rid of waste products released into the ambient water (Obi et al., 2014).

Group A (Control): Fishes were maintained in cadmium free water obtained from the University of Benin town supply.

Group B: Fishes were maintained in water tainted with exogenous *Hibiscus sabdariffa* calyx extract (HSCE), 0.25%v/v.

Group C: Fishes were maintained in water tainted with 0.3 mg exogenous cadmium.

Group D: Fishes were maintained in water tainted with 0.3 mg exogenous cadmium + HSCE 0.25%v/v.

Each group was maintained in 25 litres of water in a plastic bucket (40 litres capacity) with perforated lids. They were kept in a room with reduced illumination at 25°C. After a period of two weeks, the fish were harvested and sacrificed. The liver was excised after slitting through the abdominal region and kept in sample bottles refrigerated at -20°C until required. The serum was collected by cardiac puncture, stored in a sample plain bottle and centrifuged at 4,000 rpm for ten minutes. The resulting serum was stored at -20°C until required for biochemical assays.

Preparation of Liver Homogenates

A portion of the liver were weighed, chopped into a very small bit and homogenized in ice cold physiological saline (0.9%) using a pre-cooled mortar and pestle to obtain 20% homogenate (1:4 w/v). The homogenates were centrifuged at 4,000 rpm for 10 minutes. The resulting supernatants were separated and stored frozen at -20°C until required for biochemical assays.

2.3 Biochemical assay

Malondialdehyde (MDA) level, which is an index of lipoperoxidation was determined as thiobarbituric acid reactive substance (TBARS) as reported by Buege and Aust (1978).

Values for TBARS were quantified using a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$ and expressed in terms of malondialdehyde (MDA) units per gram tissue. Each unit represents one micromole of MDA. Catalase (CAT) activity was assayed by using the method of Cohen *et al.* (1970). Each catalase unit specifies the relative logarithmic disappearance of hydrogen peroxide per minute and is expressed as $K \text{ min}^{-1}$. Superoxide dismutase (SOD) activity was determined by the method of Misra and Fridovich (1972) and the activity was estimated and expressed as described by Baum and Scandalios (1981) in which one unit represents the amount of enzyme required for 50% inhibition of epinephrine to adrenochrome during 1 min. Total cholesterol concentration was determined by the use of cholesterol oxidase (Richmond, 1973; Allain *et al.*, 1974; Roeschlaw *et al.*, 1974). HDL- cholesterol and Triglycerides concentration was determined by the method described by National Cholesterol Education Programme (NCEP) (2001) while Low density lipoproteins (LDL and VLDL) and chylomicron fractions are precipitated quantitatively by the addition of phosphotungstic acid in the presence of magnesium ions. After centrifugation, the cholesterol concentration in the HDL fraction which remains in the supernatant were determined (Burstein *et al.*, 1970).

2.4 Statistical Analysis

The experimental results were expressed as mean \pm standard error of mean (SEM) and were subjected to one-way analysis of variance (ANOVA). The mean values were compared using Tukey's multiple comparisons tests and significant level was set at $p < 0.05$ from SPSS 16.0.

3. Results

3.1 Effect of Cadmium and *Hibiscus sabdariffa* Calyx Extract (HSCE) Treatments on Lipid Profile of the Liver of African Catfish

The effect of cadmium and *Hibiscus sabdariffa* calyx extract (HSCE) treatments on lipid profile of the liver of African catfish are presented in Table 1. There was significant ($p < 0.05$) decrease in the concentration of total cholesterol, Low Density Lipoprotein cholesterol (LDL-c) and triglycerides in HSCE (Group B) and Cd + HSCE (Group D) treated groups compared to the group treated with Cd only (Group C). Also, there was no marginal difference (p in the concentration of High Density Lipoprotein Cholesterol (HDL-c) in HSCE (Group B) and Cd + HSCE (Group D) treated groups compared to the group treated with Cd only (Group C) and the control (Group A).

3.2 Effect of Cadmium and *Hibiscus sabdariffa* Calyx Extract (HSCE) Treatments on Lipid Profile of the Serum of African Catfish

The effect of cadmium and *Hibiscus sabdariffa* calyx extract (HSCE) treatments on lipid profile of the serum of African catfish are presented in Table 2. The results showed that there was no significant ($p > 0.05$) difference in the concentration of total cholesterol and Low Density Lipoprotein cholesterol (LDL-c) when compared to the control and the group treated with Cadmium (Cd) only. However, there was significant decrease (p

< 0.05) in the concentration of triglycerides in the group treated with Cd + HSCE (Group D). More so, there was significant difference ($p < 0.05$) in the concentration of HDL-c in the group treated with Cd + HSCE (Group D) compared to Group C (Cd only) while there was no significant difference in the group treated with HSCE (Group B) only compared to Group A and Group C.

3.3 Effect of Cadmium and *Hibiscus sabdariffa* Calyx Extract (HSCE) Treatments on MDA Levels and Antioxidant Enzyme (SOD) and Catalase (CAT) Status of the Liver of African Catfish

The concentration of malondialdehyde (MDA) levels, SOD and Catalase activities in the liver of catfish are shown in Table 3. There was significant increase ($p < 0.05$) in superoxide dismutase (SOD) activities of cadmium treated group only (Group C) when compared to the control while there was marginal ($p < 0.05$) decrease in the concentration of the SOD activities in the group treated with HSCE and Cd + HSCE compared with the group intoxicated with Cd only. In addition, there was no significant difference ($p > 0.05$) in both catalase and MDA activities of the group treated with HSCE only and Cd + HSCE group when compared with group intoxicated with Cd only (Group C) and the control (Group A) in the liver of the Catfish.

3.4 Effect of Cadmium and *Hibiscus sabdariffa* Calyx Extract (HSCE) Treatments on MDA Levels and Antioxidant Enzyme (SOD) and (CAT) Status of the Serum of African Catfish

The malondialdehyde (MDA) levels, SOD and CAT activities in the fish serum are shown in Table 4. There was significant increase ($p < 0.05$) in MDA levels and SOD activities in Cd treated groups when compared to the control while there was marginal ($p < 0.05$) decrease in the concentration of SOD compared to Group C (Cd only). Also, there was no significant different ($p > 0.05$) in the activities of catalase when compared to Group C (Cd) only and control.

3.5 Effect of Cadmium and *Hibiscus sabdariffa* Calyx Extract (HSCE) Treatments on Standard Length, Total Length and Liver-Body Weight Ratio of African Catfish

The effects of cadmium and HSCE on liver-body weight ratio of fish are shown in Table 5. There was significant ($p < 0.05$) decrease in the standard length of the group treated with Cd + HSCE compared with the normal control while no significant difference was observed in Group B and Group D when compared with Group C and Group A. Also, there was significant difference ($p < 0.05$) in the total length of Group B and Group D compared with the control. On the other hand, there was no significant difference in the total length of Group B and Group D when compared to Group C. More so, in liver- body weight ratio of the group treated with HSCE only (Group B) there was significant increase ($p < 0.05$) compared with the group treated with Cd only (Group C) while no observable difference was shown in group treated with Cd + HSCE (Group D) compared with Group C. There was significant difference in Group C and Group D when compared with the control.

Table 1 Lipid Profile Assay in Liver of *C. gariepinus* Maintained in Cd Contaminated Water

	GROUP A	GROUP B	GROUP C	GROUP D
Total Cholesterol (mmol/l)	4.10±2.19 ^a	1.71±0.35 ^b	2.03±2.10 ^a	1.71±0.15 ^b
HDL Cholesterol (mmol/l)	0.75±0.31 ^a	0.89±0.17 ^a	0.86±0.16 ^a	1.27±0.48 ^a
LDL Cholesterol (mmol/l)	3.28±2.16 ^a	1.85±0.30 ^b	3.26±0.92 ^a	1.47±0.65 ^{b,c}
Triglycerides (mmol/l)	1.56±0.24 ^a	1.63±0.21 ^a	2.49±0.46 ^b	2.06±0.89 ^{a,b}

Results are expressed as Mean ± SEM (n=10). Values with different superscripts within a row are statistically significantly different from each other ($p < 0.05$).

Table 2 Lipid profile assay in Serum of *C. gariepinus* Maintained in Cd Contaminated Water

	GROUP A	GROUP B	GROUP C	GROUP D
Total cholesterol (mmol/l)	3.09±0.23 ^a	3.93±0.63 ^a	4.54±0.35 ^a	3.75±0.41 ^a
HDL cholesterol (mmol/l)	1.09±0.23 ^a	1.71±0.24 ^{a,b}	2.36±0.25 ^b	1.29±0.19 ^a
LDL cholesterol (mmol/l)	1.03±0.20 ^a	0.96±0.30 ^a	1.11±0.26 ^a	1.18±0.36 ^a
Triglycerides (mmol/l)	1.95±0.31 ^a	3.85±0.63 ^b	4.22±0.45 ^b	1.89±0.22 ^a

Results are expressed as Mean ± SEM (n=10). Values with different superscripts within a row are statistically significantly different from each other ($p < 0.05$).

Table 3 MDA Levels and Antioxidant Enzymes (SOD) and CAT) Activities in Liver of *C. gariepinus* Maintained in Cd Contaminated Water

	GROUP A	GROUP B	GROUP C	GROUP D
MDA (mol/g tissue) $\times 10^{-3}$	3.58±0.14 ^a	3.52±0.14 ^a	3.78±0.04 ^a	3.75±0.12 ^a
Catalase (K/min) $\times 10^{-1}$	5.50±0.04 ^a	5.65±0.10 ^a	5.46±0.42 ^a	5.58±0.06 ^a
SOD (units/mg tissue)	2.80±0.43 ^a	2.89±0.76 ^a	4.14±0.85 ^b	2.64±0.55 ^a

Results are expressed as Mean ± SEM (n=10). Values with different superscripts within a row are statistically significantly different from each other ($p < 0.05$).

Table 4 MDA Levels and Antioxidant Enzyme (SOD) and (CAT) Activities in Serum of *C. gariepinus* Maintained in Cd Contaminated Water

	GROUP A	GROUP B	GROUP C	GROUP D
MDA (mol/dL serum $\times 10^{-3}$)	101.45±8.22 ^a	111.87±1.32 ^a	159.83±3.01 ^b	119.79±1.05 ^a
Catalase (K/min $\times 10^{-1}$)	0.43±0.01 ^a	0.43±0.02 ^a	0.43±0.01 ^a	0.43±0.01 ^a
SOD (units/dL serum)	0.89±0.19 ^a	0.64±0.09 ^a	1.92±0.41 ^b	0.50±0.14 ^a

Results are expressed as Mean ± SEM (n=10). Values with different superscripts within a row are statistically significantly different from each other ($p < 0.05$).

Table 5 Standard Length, Total Length and Liver- Body Weight Ratio of *C. gariepinus* Maintained in Cd Contaminated Water

Treatment Groups	Standard Length (cm)	Total Length (cm)	Liver-Body Weight Ratio
A	1.66±0.343 ^a	2.09 ± 0.272 ^a	0.07±0.01 ^a
B	1.00 ± 0.234 ^{a,b}	1.08 ± 0.215 ^b	0.06±0.01 ^a
C	1.17 ± 0.248 ^{a,b}	1.49 ± 0.174 ^b	0.017±0.02 ^b
D	0.68±0.136 ^b	0.91 ± 0.142 ^b	0.02±0.01 ^b

Results are expressed as Mean ± SEM (n=10). Values with different superscripts within a column are statistically significantly different from each other ($p < 0.05$).

4. Discussion

The result of this study showed a significant decreased in the concentrations of total- and LDL cholesterol in fish maintained in Cd + HSCE tainted water and HSCE tainted water, the effect of cadmium on the lipid content was reported by earlier investigators (**Omonkhua et al., 2009; Obi et al., 2014**). The decrease in total- and LDL- cholesterol levels observed may be due to the ability of the extract to lower total- and LDL- cholesterol in the presence of cadmium in the liver of the catfish or due to a decreased de novo synthesis of cholesterol in the presence of the extract. **Chen et al. (2003)** also reported reduced total- and LDL cholesterol concentration in rats, similar to the results obtained in this study. The observed decrease in the serum triglycerides concentration and marginal increase in the serum HDL- cholesterol concentration of the catfish exposed to HSCE + Cd suggest that HSCE appears to offer protection against cadmium induced toxicity. In addition to this, the protective effect shown by HSCE extract is unlikely as a result of the mobilization of triglycerides from the breakdown of adipose tissue to meet the energy demand caused by the stressor on the fish (**Alkahem et al., 1998**). Under stress conditions, the body mechanisms are altered to combat the effect of pollutants, stressors or any foreign substances in order to maintain homeostasis in the organism. Fish under stress mobilizes triglycerides and proteins to meet an increase in demand for energy resulting from increased physical activity, biotransformation and excretion (**Alkahem et al., 1998**).

Antioxidants are substances which inhibit or delay oxidation of a substrate while present in minute concentration. Antioxidants molecules are thought to play a crucial role in counteracting free radicals induced damage to macromolecules and have been found to heal the free radical mediated damage. HSCE contains a variety of antioxidants that plays crucial role in scavenging free radicals. The observed increase in the SOD activity in liver and serum of fish exposed to Cd reveals its toxicity as a stressor in the water. The attendant increase in serum MDA level in Cd treated group appears to corroborate the observation made in this study. However, HSCE was able to act antagonistically when present together with Cd in the water. This observation could be as a result of the ability of HSCE antioxidant properties which helps to mop up free radicals in the water. This result is in agreement with the findings of **Lee, et al. (2002)** and **Tsai et al. (2002)** who reported that HSCE is a rich source of antioxidants. The increase in SOD activity could be as a result of the overwhelming effects of the fish by Cd increasing the activity of SOD and or due to the increase in the generation of reactive oxygen species by Cd competition with essential metals in protein-binding site, triggering a release of Fe^{2+} and Cu^{2+} ions similar to the report of **Adewoye and Fawole (2002)**.

The decrease in standard length, total length and liver-body weight ratio observed in Cd and Cd + HSCE treated groups suggest that the presence of the metal may be responsible for the decrease in anthropometric status of the fish which has been reported earlier (**Prabu et al., 2012**). The significant decrease in liver-body weight ratio of catfish exposed to Cd and Cd + HSCE observed in this study is in consonance with the report that the presence of pollutants such as heavy metals in water reduces organs weight especially the liver which is the main site of detoxification of toxicants (**Chen et al., 2012**). **Pavlovic et al. (2010)** reported that the response of fish to a variety of metal and organic pollutants are transient and are dependent on the species, enzymes and single or mixed contaminants. This may be due to the fact that under oxidative stress, the toxic effect of pollutants may overwhelm the antioxidant defense mechanism of the fish; hence the fish used energy to overcome stressful environmental conditions instead of developmental and biological activities (**Adeogun, 2012**).

5. Conclusion and recommendation

It was evidence from the findings of this study that aqueous extract of *Hibiscus sabdariffa* (HSCE) has potential to lower high total cholesterol, triglycerides and LDL-c and increase low HDL-c in cadmium intoxicated catfish. Also, it possesses the capability to protect cells/tissues of catfish from free radicals' damage and oxidative stress. Hence, the plant could therefore be exploited in agriculture for the sustainability of fish population in polluted aquatic systems. Further study is hereby recommended to clarify the mechanism behind the decrease in the anthropometric status observed in these fish when exposed to cadmium.

Declaration of interest

The authors have no conflicts of interest. All authors give their consent to publication with this journal. The authors alone are responsible for the content and writing of the paper.

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