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Effect of Aqueous Extract of *Hibiscus Sabdarifa* on Cadmium Induced Biochemical Changes in Brain of *Clarias Gariepinus*

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Abstract: **The effect of *Hibiscus sabdariffa* calyces extract on cadmium (Cd) induced biochemical changes in the brain of *Clarias garipenus* was studied. Forty (40) juveniles African catfish (*Clarias gariepinus*) were used for the study. Test group was administered 0.3 mg/l Cd for two weeks. The activities of superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) formation were determined, phytochemical screening of plant extract and histopathology examination of brain tissue was also carried out. Phytochemical screening of *Hibiscus sabdariffa* calyces revealed the presence of Alkaloids, Carbohydrate, Amino acids, Saponin, Proteins, Glycoside, Flavonoids, Tanins and Phenol while Anthraquiones, Steroids and Sterols were absent. There was no significant difference in MDA levels and SOD activity of all test groups when compared to the control, but a slight decrease ($p<0.05$) was seen in the CAT activity. Histopathological observation on the brain showed that there was discolouration on the brain of fish exposed to Cd, mild degenerative changes were also observed. The Environment is constantly invaded with pollutants, increasing the risk of cellular damage from reactive oxygen species (ROS), *Hibiscus sabdariffa* calyces extract is rich in antioxidants thus it can protect cells/tissues from free radical damage and oxidative stress.**

Keywords: Hibiscus sabdariffa, Clarias garipenus, **Superoxide dismutase, Catalase, Malondialdehyde.**

1. INTRODUCTION

Exposure to a countless number of toxic substances and compounds such as mercury, cadmium, lead, copper, arsenic, air pollutants, pesticides, plastics, cigarette smoke, diesel fumes and nano-particles are a major threat to the health of ecosystems, this is because of the high toxicity conferred by their persistent nature in the environment. Heavy metals play a prominent role in causing serious health hazard in ecosystems and organisms [8]. Their presence in aquatic environment is brought about by direct or indirect agricultural and industrial discharges, the fact that heavy metals cannot be destroyed through biological degradation and have the ability to accumulate in the environment makes these toxicants deleterious to the aquatic environment and consequently to humans who depend on aquatic products as food source [20], [13]. Fish are mostly being used for the assessment of the quality of aquatic environment, as such they can serve as bio-indicators of environmental pollution [11]. African catfish (*Clarias gariepinus*) is of enormous commercial importance because it is the most common fresh water fish widely consumed in Nigeria [13]. It can therefore be a good model to study responses to various environmental contaminants.

Hibiscus sabdariffa linn (roselle) is an annual dicotyledonous herbaceous shrub plant popularly known as "zobo" in Nigeria. It's been reported that extract from the red calyces of *Hibiscus sabdariffa* contains potent antioxidant principles [20], [14]. Lots of research has been carried out on the effect of heavy metal toxicity on various parts of the fish, but little has been done on the brain tissue, hence the rationale behind this present study. The aim of this study was to investigate the effect of aqueous extract of *Hibiscus sabdarifa* calyx on the biochemical changes induced by cadmium in the brain of African catfish (*Clarias gariepinus*).

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2. MATERIALS AND METHOD

Forty (40) juveniles African catfish (*Clarias gariepinus*) were bought from the Department of Environmental Biology and Fisheries, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria and were acclimatized in the laboratory for two weeks before the commencement of the study. Regular feeding of fish was done at 3% of the total body weight twice daily. Regular change of water was done every other day to maintain the biochemical oxygen demand and to get rid of waste products released into the water. The fishes were grouped into four groups (A to D) of ten fish each on the basis of their mean body weight. Group **A**: The control group maintained on normal fish feed (coppens) Group **B**: Test group administered 0.3 mg/l Cd Group **C**: Test group administered 0.3 mg/l Cd and treated with *Hibiscus sabdariffa* calyx extract, 0.25% (v/v). Group **D**: Test group administered *Hibiscus sabdariffa* calyx extract, 0.25% (v/v).

Aqueous extract of *Hibiscus sabdariffa* was prepared by boiling 160 g the calyces in 200 ml of distilled water at 100°C for 15 min and left standing for 48 hours. After 48 hours, the aqueous extract was separated by filtration. Gravimetric analysis showed that 1 ml of the aqueous extract contained 0.04 g (40 mg) of solid residue. The filtrate was kept in plastic bottle and stored in a refrigerator at 4 °C until time of use. Stock and test solutions were also prepared. After two weeks of exposure, the fishes were sacrificed and the brain excised, they were weighed and homogenized in ice-cold normal saline yielding 20% homogenate in a pre-cooled mortar and pestle. The homogenates were centrifuged at 4000 rpm for 10 minutes. The supernatants were separated and stored frozen at -20°C until required for the biochemical assays.

Biochemical Assay:

Brain tissue homogenate were analyzed for Malondialdehyde (MDA), Superoxide dismutase (SOD) activity, and Catalase (CAT) activity by the method of [4], [12], and [3]. respectively.

Histopathology:

Brain tissue were collected and fixed in 10 % (v/v) formaldehyde, dehydrated through ascending grades of ethanol, cleaned in xylene, and processed into paraffin blocks. Tissue sections (5 µm thick) were prepared according to the method described by [5] and stained with hematoxylin and eosin. The photomicrographs were examined using light microscopy for demonstration of pathological changes.

Phytochemical screening of *Hibiscus sabdariffa* calyces:

The Phytochemical screening of *Hibiscus sabdariffa* calyces was carried out by the method described by [18].

Statistical Analysis:

All studies were carried out in triplicates, experimental results were expressed as mean ± standard deviation (SD), data were analyzed by one way analysis of variance (ANOVA), followed by paired student's t-test. Values were considered statistically significant at $P < 0.05$.

3. RESULT AND DISCUSSION

TABLE 1: Phytochemical screening of *Hibiscus sabdariffa* calyces

Saponin	+
Flavonoids	+
Amino acids	+
Alkaloids	+
Anthraquiones	-
Glycoside	+
Carbohydrate	+
Tanins	+
Proteins	+
Steroids	-
Phenol	+
Sterols	-

Present = + Absent = -

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TABLE 2: Brain weight and growth characteristics

	Brain Weight	Standard Length	Total Length
Group A	0.32 ± 0.11^{a}	21.56 ± 3.24^{a}	24.72 ± 3.58^{a}
Group B	0.39 ± 0.13^{a}	20.67 ± 2.11^{a}	23.61 ± 2.38^{a}
Group C	0.31 ± 0.18^{a}	20.45 ± 2.38^{a}	23.10 ± 4.23^{a}
Group D	0.33 ± 0.16^{a}	20.96 ± 2.63^{a}	24.32 ± 3.82^{a}

Values are means (±SD) of triplicate determinations, n = 10.

TABLE 3: MDA Levels and Antioxidant Enzyme Activities

	MDA(units/g tissue)	Catalase ($Kmin^{-1}$) x 10^{-1}	SOD (units/mg tissue) x 10^{-2}
Group A	251.20 ± 1.46^{a}	4.30 ± 0.01^{a}	6.36 ± 3.24^{a}
Group B	106.30 ± 1.39^{a}	$4.28 \pm 0.02^{a,b}$	6.74 ± 2.44^{a}
Group C	258.00 ± 0.86^{a}	4.23 ± 0.02^{c}	4.64 ± 2.71^{a}
Group D	252.09 ± 1.29^{a}	4.24 ± 0.01^{a}	5.02 ± 2.62^{a}

Values are means (±SD) of triplicate determinations, n = 10.

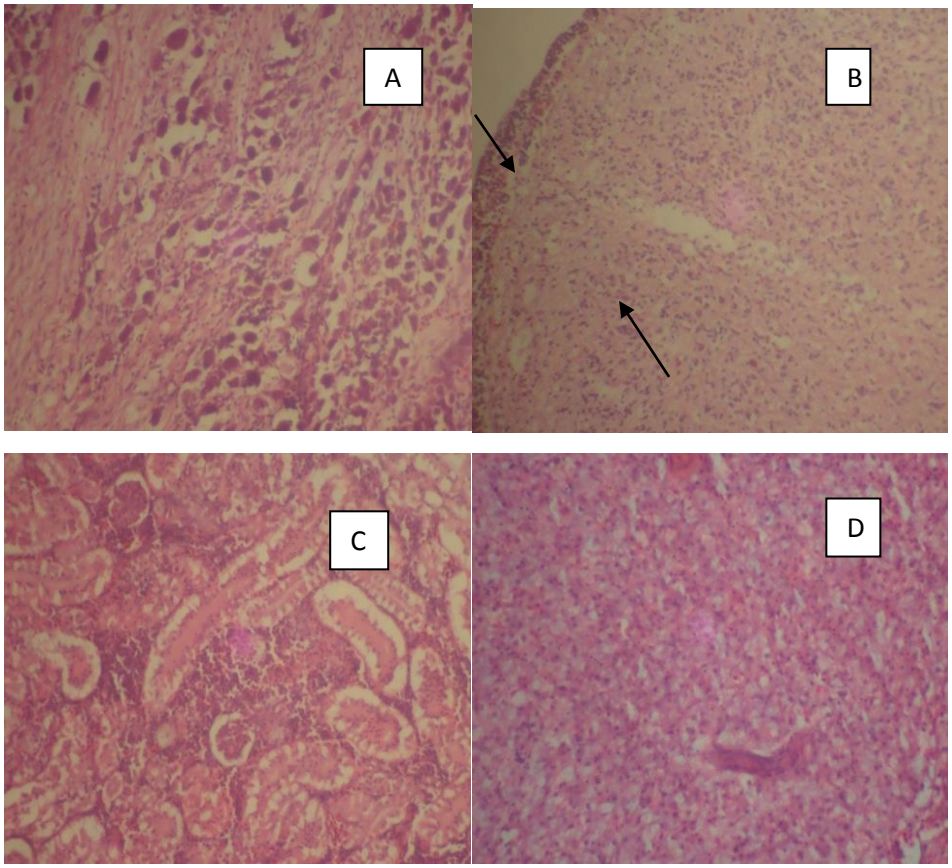

Fig 1 : Photomicrographs (x 100) of brain tissues of *Clarias gariepinus* fingerlings A = Group A, B = Group B, C = Group C, D = Group

Table 1 presents the result of the phytochemical screening of Hibiscus sabdariffa calyces, Alkaloids, Carbohydrate, Amino acids, Saponin, Proteins, Glycoside, Flavonoids, Tanins and Phenol were present while Anthraquiones, Steroids and Sterols were absent. Table 2 shows the Brain weight and growth characteristics of the fish, statistically; there was no significant difference ($p>0.05$) in test group and control. Brain MDA Levels and antioxidant enzyme activities of the fish is presented in table 3, there was no significant difference in MDA levels and SOD activity, but a slight decrease ($p<0.05$) was seen in the CAT activity of the group C fishes when compared to the control and other groups. Histopathological examination of the brain at the end of the experiment are depicted in Figure 1. The control group showed normal architecture of the brain tissues (Plate A). Fishes exposed to Cd showed mildly degenerated changes (Plate B).

Discussion:

Environmental pollutants induce oxidative stress by generating free radicals (reactive oxygen species). High level of reactive oxygen species (ROS) in tissues may lead to cellular damage. Antioxidants (enzymatic and non – enzymatic) protects cellular systems from damages done by these free radicals. Cadmium is a heavy metal that generates ROS, it enters the electron transport chain in mitochondria, leading to accumulation of unstable semiubiquinones which donate electrons and create superoxide radicals. Cadmium also affects antioxidant enzymes, especially SOD and CAT, and is able to displace copper and iron in various proteins, freeing these metals to then participate in the Fenton reaction [6].

The result of the phytochemical screening of *Hibiscus sabdariffa* calyces, showed that it contains a variety of antioxidants, this is in agreement with works of [10]. The brain is very susceptible to oxidative damage through free radicals as it contains high amounts of unsaturated lipids and utilizes about 20% of total oxygen demand of the body. Catalase (CAT) is an important enzymatic antioxidant, whose activity is generally low in the brain but highly active in other tissues. In this study, the specific activity of brain CAT decreased in group C, treatment of Cd contaminated water with *Hibiscus sabdariffa* calyces extract (which is rich in antioxidants) mops up free radicals which may have been initiated by Cd. This result is in agreement with the findings of [20], [19] and [1], who reported that *Hibiscus sabdariffa* calyces extract is a rich source of antioxidants.

Malondialdehyde (MDA) is one of the oxidative damage products of lipid peroxidation. Its presence in tissues indicates oxidative stress. This present study showed no significant difference in MDA levels of all test groups compared with the control, this may be attributed to the dose of the contaminant and the duration of exposure. Although, most study reports an increase in MDA level of fishes [13], [9]. The activity of antioxidant enzymes may be normal, enhanced or inhibited under chemical stress depending on the intensity and the duration of the stress applied, and the susceptibility of the exposed species. The activity of SOD was not affected in all test group and control.

[16] reported that the response of fish to a variety of metal and organic pollutant or foreign substance are transient and are dependent on the species, enzymes and type of pollutant or foreign substance. The histopathological observation on the brain showed that there was discolouration on the brain of fish exposed to Cd, this finding is in agreement with results of [15], [2],[17] and [7], who reported degenerative changes in fish exposed to various pollutants and contaminants.

4. CONCLUSION

The Environment is constantly invaded with pollutants and contaminants resulting from human activities, climate change, chemical and non- human activities, thus, increasing the risk of cellular damage from reactive oxygen species (ROS). Constant exposure to these pollutant places human and animals at risk, making them vulnerable to various health hazards. Plants are rich sources of antioxidants, *Hibiscus sabdariffa* calyces extract is very rich in antioxidants thus it can protect cells/tissues from free radical damage and oxidative stress, consequently, it can be exploited for pharmacological and neutraceutical advantages.

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