Scientific Research Publishing

Advances in Biological Chemistry, 2020, 10, 43-54
https://www.scirp.org/journal/abc
ISSN Online: 2162-2191
ISSN Print: 2162-2183

Physico-Chemical Evaluation and Hematological Responses in *Clarias gariepinus* (Burchell 1822) Juveniles Exposed to *Adenium obesum* Stem Bark Acute Bioassay

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How to cite this paper: Muyiwa, B.O., Emmanuel, N.S., Yahuza, F.M., Aliyu, M., Inegbenosun, C.U. and Musah, I. (2020) Physico-Chemical Evaluation and Hematological Responses in *Clarias gariepinus* (Burchell 1822) Juveniles Exposed to *Adenium obesum* Stem Bark Acute Bioassay. *Advances in Biological Chemistry*, **10**, 43-54.
https://doi.org/10.4236/abc.2020.102004

Received: August 23, 2019
Accepted: March 27, 2020
Published: March 30, 2020

Abstract

The experiment was conducted to evaluate the acute toxicity of aqueous extract of *Adenium obesum* stem bark in juveniles of *Clarias gariepinus* by assessing the hematological responses of the exposed groups compared to the control in a static non-renewal bioassay for 96 hours. The fishes (N = 180, mean weight and length 21.48 ± 3.32 g and 11.37 ± 1.23 cm) respectively were randomly distributed 10 (ten) fishes per group in triplicates constituting six experimental groups as follows; G1 (Control), G2 (6.5 mg/l), G3 (7.8 mg/l), G4 (8.5 mg/l), G5 (9.5 mg/l) and G6 (11.5 mg/l). Red blood cell (RBC) count, packed cell volume (PCV) and hemoglobin concentration were decreased significantly ($P < 0.05$) as opposed to the significant ($P < 0.05$) increase in total white blood cell count (TWBC). Thus the study concludes that aqueous extract of *Adenium obesum* stem bark is toxic to erythrocytes while a converse adaptive response in the white blood cells.

Keywords

Adenium obesum, *Clarias gariepinus*, Hematology, Toxicity

1. Introduction

Hematological parameters are indicators of stress and give information on the physiological responses of fishes to a changing external environment [1]. It has

been used as an index of fish health status in a number of fish species to detect physiological changes, as a result of exposure to different stressful conditions such as handling, pollutants, metals, hypoxia, anesthetics and acclimatization [2] [3] [4]. Blood of living organisms is very sensitive to changes and is widely used in ichthyology and aquaculture researches as well as toxicology and biological monitoring [5] [6] [7]. Its composition often reflects the total physiological condition [8]. Because blood is in direct contact with various organ and tissues of the body, it is therefore the medium of intercellular and intracellular transport from which the physiological state of an animal at a particular time can be extrapolated. Thus, blood provides an ideal medium for toxicity studies. The blood parameters have been considered as diagnostic indices of pathological condition as well as important markers for the assessment of systemic functions and overall health of animals. Furthermore, it also helps in diagnosing the structural and functional status of animals exposed to the toxicant [9] [10]. It is important in toxicological research because a hematological alteration is a good method for rapid evaluation of the chronic toxicities of a compound, a thin epithelial membrane separates fish blood from the water and any unfavorable changes in the water medium which is reflected in the blood of the fish [11] [12] [13]. *Oreochromis niloticus* exposed to cadmium showed significant reduction in RBC, Hb and PCV [14]. Although the hematological response to the toxicity of ethanol extract of *Adenium obesum* stem bark has been reported by [15], the study is however not carried out in fishes. Therefore this study seeks to investigate the hematological response in Juvenile *Clarias gariepinus* exposed to aqueous extract of *Adenium obesum* stem bark in an acute bioassay.

2. Materials and Methods

2.1. Plant Collection

The stem bark of *Adenium obesum* was harvested from Bassawa area within Zaria, Kaduna State in Nigeria during the November-December season, 2016, and authenticated at the Herbarium section of the Department of Biological Sciences, A.B.U, Zaria, by Mallam Namadi sunusi, where a specimen was deposited and a voucher number 01386 was assigned. The stem bark was picked and dried under shade until constant weight was obtained.

2.2. Plant Extraction

The extraction was carried out as described below according to the method of [16]. The stem bark of *Adenium obesum* was dried under shade until constant weight is obtained, stem bark were crushed into coarse powder using a pestle and mortar and stored for the extraction process. The fine powder was added into distilled water and shaken gently for ten minutes using a shaker to make a homogenous mixture. The mixture was left for 24 hours after which it was filtered and the filtrate used for the study.

2.3. Experimental Site

This study was conducted in the department of Veterinary Pathology, Faculty of Veterinary medicine, Ahmadu Bello University Zaria, Nigeria.

2.4. Ethical Approval and Experimental Animals

Ethical clearance approval was obtained from Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) with approval number ABUCAUC/2017/014. One hundred and eighty (180) live juvenile African-sharp tooth catfish (*C. gariepinus*), average weight and weight of (21.48 ± 3.32) g and length of (11.37 ± 1.23) cm respectively were sourced from a commercial catfish farm (fish house), located within Kaduna State in Nigeria, and the species determined at the fishery section, Department of Biological Sciences, ABU Zaria, Nigeria. Fish acclimatization lasted for 21 days under natural day and night photo-periods (12/12-h) with complete changing of pond water once in every three days. The fish were fed to their satisfaction (ad libitum) twice daily with 2 mm Coppens® fish feed for aquaculture (Coppens® International by Helmond, Holland).

2.5. Experimental Design

A total of one hundred and eighty (180) fishes with an average mean body weight of 21.48 ± 3.32 gram and an average mean length of 11.37 ± 1.23 cm were divided into six (6) groups of 10 fishes per group in triplicate after a 14 days acclimatization period. Fishes were then selected at random and kept in a static system of water. Feeding was halted a day prior to exposure to aqueous extract *Adenium obesum* stem bark throughout the test period, both for range finding and definitive test.

2.6. Toxicity Bioassay

A range finding test to determine the five extract concentrations as described by [17] was performed. Mortality was used as an end point of toxicity and this was determined according to [18]. A preliminary range finding test was carried out based on the concentration of the active ingredient in the test chemical. The range finding was done using the following concentrations as described by [17]. The result obtained from the range finding test provided a guide for the definitive test. Definitive test was carried out using six groups, G1 (control), G2 (6.5 mg/l), G3 (7.8 mg/l), G4 (8.5 mg/l), G5 (9.5 mg/l) and G6 (11.5 mg/l). The acute toxicity tests were performed according to the static non-renewable bioassay procedure [19]. The experimental design consisted of a control and five concentrations of the extract, (6.5 mg/l, 7.8 mg/l, 8.5 mg/l, 9.5, and 11.5 mg/l) with 10 (ten) fish per group in triplicate. A glass aquarium of 40 litres capacity with aeration was used per each group. Each glass aquarium was covered with nylon mesh tied firmly with rubber strap to prevent the fish from jumping out. Fish showing no respiratory movement and response to tactile stimuli were considered as dead and removed immediately. During the exposure in different con-

centrations of aqueous extract *Adenium obesum* stem bark, the behavioural changes of the fishes were also recorded. Daily physicochemical characteristics, temperature and pH of fish culture water were ascertained using a Hanna "Combo portable hand instrument (Hi 98129, Hanna Instrument, Mauritius) while their dissolved oxygen contents were similarly established using the modified Winkler-Azide method [20] [21].

2.7. Collection of Blood Samples

Two (2) ml of blood was collected from each sampled fish via caudal veno-puncture, using the lateral approach, a 25 G needle was introduced through the lateral line laterally to the body of the fish until contact was made with the spine at which point the needle was gently withdrawn with aspiration. As the blood flowed into the needle the gentle backward withdrawal was halted allowing for more blood to be drawn from which about 1 ml of the collected blood was dispensed into a bottle containing ethylene diamine tetra acetic acid (EDTA) anticoagulant for heamatological evaluations.

2.8. Hematological Assessment

The packed cell volume (PCV) was determined using the standard method described by [22]. The red blood cells (RBC) and the total white blood cells (TWBC) were determined using the method described by [23]. The haemoglobin concentration was determined using the method described by [24]. The differential white blood cells were determined using the method described by [25].

2.9. Statistical Analyses

Data was expressed as mean ± SEM and then subjected to Two-way Analysis of Variance (ANOVA). Values with $P < 0.05$ were considered statistically significant. Tukey's multiple comparison tests for means was used to compare differences between the various means using SPSS version 20.

3. Results

3.1. Result of Physicochemical Parameters Following Exposure to Aqueous Extract *Adenium obesum* Stem Bark

Table 1 shows the result of some physicochemical parameters. Results in this study showed a non-significant ($P > 0.05$) increase for the PH across the groups. Although the changes observed in the oxygen and temperature were also non-significant ($P > 0.05$), however there was a significant ($P < 0.05$) dose dependent increase in the total dissolved solids (mg/l) and electric conductivity (µs/cm).

3.2. Result of Red Blood Cell count, Hemoglobin Concentration, Packed Cell Volume, Mean Corpuscular Volume and Mean Corpuscular Hemoglobin Following Exposure to Aqueous Extract *Adenium obesum* Stem Bark

There was a significant ($P < 0.05$) decrease in the Red Blood Cell (RBC) level in

all the exposed groups compared to control with a corresponding increase in the concentration of the aqueous extract *Adenium Obesum* stem. The result of Hb shows a statistically significant decrease ($P < 0.05$) in all the treated groups compared to the control; G2 (9.98 ± 0.01 g/dl), G3 (9.90 ± 0.00 g/dl), G4 (9.44 ± 0.00 g/dl); G5 (9.14 ± 0.00 g/dl) and G6 (8.18 ± 0.16 g/dl) vs G1 (11.01 ± 0.01 g/dl). PCV in Table 2 shows a significant decrease in all the treated groups compared to the control thus; 27.02% ± 0.01%, 25.02% ± 0.01%, 22.01% ± 0.00%, 20.04% ± 0.01%, and 17.67% ± 0.33% for G2, G3, G4, G5 and G6 respectively versus G1 (32.33% ± 0.67%). This result is also consistent with the changes observed in MCV and MCH. There was a significant ($P < 0.05$) decrease in MCV of all the exposed groups compared to the control thus; G2 (130.07 ± 0.01 μm^3), G3 (127.07 ± 0.58 μm^3), G4 (121.00 ± 0.57μm^3), G5 (117.44

Table 1. Physicochemical parameters of the different concentrations for acute toxicity test.

GROUP	PH	TEMP (˚C)	DO(mg/l)	TDS (mg/l)	Electric Conductivity (µs/cm)
G1	7.01 ± 0.01^b	27.01 ± 0.01^a	4.87 ± 0.01^a	3.37 ± 0.01^a	35.02 ± 0.12^a
G2	8.00 ± 1.01^b	27.02 ± 0.01^a	4.88 ± 0.03^a	46.01 ± 3.01^b	124.22 ± 1.42^b
G3	8.01 ± 0.11^b	27.00 ± 0.00^a	4.79 ± 0.12^a	53.02 ± 3.22^c	231.10 ± 2.67^c
G4	8.02 ± 0.01^b	27.00 ± 0.00^a	4.89 ± 0.11^a	77.13 ± 6.13^d	354.22 ± 4.11^d
G5	8.07 ± 0.02^b	27.00 ± 0.00^a	4.91 ± 0.20^a	86.05 ± 7.55^e	397.11 ± 4.87^e
G6	8.13 ± 0.01^b	27.01 ± 0.02^a	4.88 ± 0.03^a	107.02 ± 9.65^f	499.81 ± 6.31^f

Data are presented as mean ± SEM. KEYS: G1 (Control), G2 (6.5mgl), G3 (7.8 mg/l), G4 (8.5 mg/l), G5 (9.5 mg/l), G6 (11.5 mg/l); TEMP = temperature, DO = dissolved oxygen, TDS = total dissolved solids. Values in each column with different superscripts are significantly different at $P < 0.05$.

Table 2. Red Blood Cell count, Hemoglobin Concentration, Packed Cell Volume, Mean Corpuscular Volume and Mean Corpuscular Hemoglobin Following 96-hr Exposure to Aqueous Extract *Adenium obesum* Stem Bark.

TREATMENT GROUPS	ACUTE BIOASSAY HEMATOLOGICAL PARAMETERS				
	RBC ($\times 10^{12}$/L)	Hb (g/dl)	PVC (%)	MCV (μm^3)	MCH (pg)
G1	2.73 ± 0.03^a	11.01 ± 0.01^a	32.33 ± 0.67^a	134.84 ± 0.01^a	33.28 ± 0.57^a
G2	2.06 ± 0.01^b	9.98 ± 0.01^b	27.02 ± 0.01^b	130.07 ± 0.01^b	29.47 ± 0.11^b
G3	1.33 ± 0.02^c	9.90 ± 0.00^b	25.02 ± 0.01^c	127.07 ± 0.58^c	22.15 ± 0.04^c
G4	1.05 ± 0.03^d	9.44 ± 0.00^c	22.01 ± 0.00^d	121.00 ± 0.57^d	20.23 ± 0.01^d
G5	0.97 ± 0.04^e	9.14 ± 0.00^d	20.04 ± 0.01^e	117.44 ± 1.16^e	18.35 ± 0.01^e
G6	0.91 ± 0.01^f	8.19 ± 0.16^e	17.69 ± 0.33^f	111.02 ± 0.57^f	14.7 ± 0.34^f

Data are presented as means ± SEM. KEYS: G1 (Control), G2 (6.5 mgl),G3(7.8 mg/l), G4 (8.5 mg/l), G5 (9.5 mg/l), G6 (11.5 mg/l); Values in each column with different superscripts are significantly different at $P < 0.05$.RBC,red blood cell; Hb, Hemoglobin Concentration; PVC, Packed Cell Volume; MCV, Mean Corpuscular Volume; MCH, Mean Corpuscular Hemoglobin.

± 1.16 μm^3) and G6 (111.02 ± 0.57 μm^3) versus G1 (134.84 ± 0.01 μm^3). The Mean Haemoglobin Concentration (MHC) levels of the groups G2, G3, G4, G5 and G6 (29.47 ± 0.11 pg, 22.15 ± 0.04 pg, 20.23 ± 0.00 pg, 18.35 ± 0.01 pg, 14.70 ± 0.33 pg) respectively decreased significantly ($P < 0.05$) compared to the control (33.27 ± 0.57 pg).

3.3. Result of Mean Corpuscular Hemoglobin Concentration, Total White Blood Cell Count, Heterophil, Eosinophil, Monocytes and Lymphocyte Following Exposure to Aqueous Extract *Adenium obesum* Stem Bark

There was a significant decrease ($P < 0.05$) in the mean corpuscular hemoglobin concentration (MCHC) in all the treated groups compared to the control thus; G2 (27.67 ± 0.03 g/dl), G3 (26.67 ± 0.03 g/dl), G4 (24.90 ± 0.06 g/dl), G5 (24.25 ± 0.01 g/dl) and G6 (23.01 ± 0.33 g/dl) vs G1 (33.28 ± 0.5 g/dl) respectively as shown in Table 3. Total white blood cell (TWBC), Heterophil (He), monocytes (mon) and Lymphocytes (Lym) were significantly ($P < 0.05$) increased in all the exposed groups compared to the control group as shown in Table 3 except the level of He in G2 and Eos in G3 where the changes were not significant ($P > 0.05$) compared to the control.

4. Discussion

Physico-chemical parameters such as temperature, pH, dissolved oxygen, pH, electric conductivity and total dissolved solids are vital aquatic indices which determine fish health, growth and reproduction [26]. The increase in pH with time could have been due to the production of basic products of metabolism which precipitated an increase in the acidity. This study is however converse to that [27] in the acute bioassay of *Clarias gariepinus* exposed to sponge plant fruit

Table 3. Mean Corpuscular Hemoglobin Concentration, Total White Blood Cell Count, Heterophil, Eosinophil, Monocytes and Lymphocyte following 96-hr Exposure to Aqueous Extract *Adenium obesum* Stem Bark.

GROUP	ACUTE BIOASSAY HEMATOLOGICAL PARAMETERS					
	MCHC (g/dl)	TWBC (×10^9/L)	He (%)	Eos (%)	Mon (%)	Lym (%)
G1	33.28 ± 0.57^a	1.98 ± 0.01^a	5.34 ± 0.06^a	2.67 ± 0.03^a	14.20 ± 0.06^a	77.67 ± 0.33^a
G2	27.67 ± 0.03^b	2.13 ± 0.01^b	5.66 ± 0.01^a	2.37 ± 0.01^b	14.51 ± 0.01^b	78.88 ± 0.34^b
G3	26.67 ± 0.03^c	2.67 ± 0.01^c	5.94 ± 0.01^b	2.65 ± 0.01^a	14.82 ± 0.01^c	80.91 ± 0.54^c
G4	24.90 ± 0.06^d	2.53 ± 0.01^d	5.98 ± 0.01^b	2.98 ± 0.01^c	15.02 ± 0.01^c	82.79 ± 0.30^d
G5	24.25 ± 0.01^d	2.72 ± 0.01^e	6.05 ± 0.01^b	3.28 ± 0.01^d	15.16 ± 0.01^c	84.88 ± 0.34^e
G6	23.01 ± 0.33^e	2.92 ± 0.01^f	6.45 ± 0.23^c	3.43 ± 0.21^e	15.67 ± 0.14^c	87.71 ± 0.86^f

Data are presented as means ± SEM. KEYS: G1 (Control), G2 (6.5 mgl), G3 (7.8 mg/l), G4 (8.5 mg/l), G5 (9.5 mg/l), G6 (11.5 mg/l); Values in each column with different superscripts are significantly different at $P < 0.05$. MCHC = Mean Corpuscular Hemoglobin concentration, TWBC = total white blood cell count, He = heterophil, Eos = Eosinophil, Mon = monocyte, Lym = lymphocyte.

extract which reported a low pH. TDS and Electrical conductivity also increased across the different treatments which may be due to the chemical composition of *Adenium obesum* [28] DO is one of the most important factor for all living organisms especially fish survival (Bartram and Balance, 1996). DO was maintained steadily in this study as opposed to the reports of [29] which reported a decrease in DO. The result of DO according to this study could be attributed to the continuous aeration provided by the aerators within the system. The physico-chemical parameters monitored in this study seemed to have contributed little or none to the toxicity of *Adenium obesum* stem bark extract on the blood cells.

Hematological evaluation is a pathophysiological indicator of the state of health of the whole body, therefore, the hematology profile is important in diagnosing the structural and functional status of fish exposed to contaminants [30]. The resultant decrease in the RBC in this study could have been from a possible deleterious effect of the extract on the cell membrane; compromising the structural integrity of the lipid bi-layer of the membrane consequently culminating in hemolysis. Other possible pathology would include impairment of hematopoietic tissues as well as synthesis related factors. Stress related complications could have contributed to the hemolysis of the cells precipitated by generation of reactive oxygen species which are known provoking factors for lipid peroxidation. However the increase observed in the white blood cell series in this study could be from a possible adaptive immune response. These results agree with that of [31] who reported a reduction in RBC, HB and PCV in fish exposed to ethanol extract of *Adenium Obesum* stem bark. Similar studies on the effect of plant extracts on hematological responses was conducted by [32] who also reported a reduction in the RBC, PCV and Hb levels in *Clarias gariepinus* fingerlings exposed to the roots of *Telfairia occidentalis* [33] study on white tilapia *oreochromis niloticus* (Trawavas) reported a reduction in the levels of RBC, Hb, and PCV on exposure to almond (*Terminalia catappa)*, pawpaw (*Carica papaya)*, Neem (*Azadirachta indica)*, Tobacco (*Nicotiana tobacum)* and Cassava (*Manihot esculenta*) extracts. [34] [35] [36] reported a reduction in all the blood indices of *Clarias gariepinus* fingerlings exposed to Tobacco leaf except MCHC that showed a decrease. The reduction of RBC, Hb, PCV and erythrocytic indices in this study also agreed with the findings of [37]-[46]. Reductions in hemoglobin, packed cell volume and red blood counts were reported in fish exposed to toxicants, [47] who reported changes in hemoglobin percentage and red blood cells count of the fish *Clarias batrachus.* The reduction in PCV is suggestive of anemia as it is the variable that is normally used to assess the basic status of the erythrocyte [48]. The pan leukocytosis recorded in the exposed groups could be due to an attempt by the fish to fight against the toxicant (plant extract) which has led to the production of more antibodies (WBC) to improve the health status of the fish. Similar findings were reported by [49] that the increase in WBC during acute and sub-lethal treatment may be due to stimulated lymph myeloid tis-

sue as a defense mechanism of the fish to tolerate the toxicity. Enhancement in the total white blood cell count following exposure in the exposed groups could be possible due to leucocytosis which is an outcome of proliferation of hemopoietic cells, leading to increase of the TWBC in the peripheral blood [50] [51]. Changes in WBC and differential count have been reported to play important roles in the assessment of the state of health of fishes, and leucopenia and leukocytosis has been reported in the fish, *Clarias gariepinus* exposed to pathogens, heavy metals and chemotherapeutants [52] [53].

5. Conclusion

The observed reduction in the Packed Cell Volume (PCV), Hemoglobin Concentration (Hb) and Red Blood Cells (RBCs) values in the exposed fish may either be due to impaired erythropoietic response or increased destruction of RBCs thus implying that the extract of the aqueous extract of *Adenium obesum* could caused anemia. The leukocytosis observed is likely due to the adaptive immune response of the fish to protect itself against the toxicant in this case, the aqueous extract of *Adenium obesum* stem bark. From the hematological profile findings in this study the *Adenium obesum* extract can be said to be toxic to fish or aquatic animals red blood cells if not used within its safety or sub lethal concentration with great care and discretion in researches or studies.

Acknowledgements

This is to recognize the contribution of Professor Sani Adamu, Professor of Clinical Pathology, Department of Veterinary Pathology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, for his guidance and analysis of the hematological results.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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