Original Article

Sahel Journal of Veterinary Sciences

Sahel J. Vet. Sci. Vol. 22, No. 4, Pp 1-10 (2025)
https://doi.org/10.54058/zdra0r42

Article History
Received: 26-08-2024
Revised: 16-03-2025
Accepted: 13-12-2025
Published: 13-01-2026

# Ameliorative Effects of *Borreria verticillata* aqueous extract on *Adenium obesum Stem Bark* aqueous *extract* induced toxicity on the Hematological Profile of *Clarias gariepinus* (Burchell 1822) juveniles

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**ABSTRACT**

The present study was carried out to determine the effect of *Borreria verticillata* aqueous extract on *Adenium obesum* stem bark extract-induced hematological alterations in juveniles of Catfish. The study consisted of one hundred and fifty (150) *Clarias gariepinus* juveniles randomly distributed into ten (10) groups of fifteen (15) fish per group. G1 (control) received no treatment, Groups 2, 3, 4and 5 received 25, 50, 100, 200 mg/L of the *Borreria verticillata aqueous extract* (BVAE) respectively for 28 days. Group 6 received 0.838 mg/L of *Adenium obesum extract* (AOE) only for 28 days, which is 10%$LC_{50}$ of AOE. Groups 7,8,9 and10, received the combinations of the extracts, first the 10%$LC_{50}$ AOE followed by 25 mg/L BVAE, 50 mg/L BVAE, 100 mg/L and 200 mg/L BVAE, respectively). Red blood cell (RBC), hemoglobin concentration (Hb) and packed cell volume (PCV) were significantly ($P < 0.05$) lower in Group 6 (10% $LC_{50}$ AOE) when compared to the corresponding values in the groups (7-10) exposed to AOE and treated with BVAE. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were significantly ($P < 0.05$) lower in group 6 when compared to the groups (7-10) that were exposed to AOE and treated with BVAE. The total white blood cells count was significantly ($P<0.05$) higher in group 6, compared with the values in the groups (7-10) that were exposed to AOE and treated with BVAE. The finding from this study showed that *Borreria verticillata aqueous extract* ameliorated the hematological alterations in *Clarias gariepinus* induced by *Adenium obesum* stem bark extract toxicity, whereas exposure to BVAE alone did not illicit any hematological alterations.

**Keywords:** *Adenium obesum*; *Borreria verticillata; Clarias gariepinus;* Hematology

## INTRODUCTION

Aquaculture is a rapidly growing agricultural economic sub sector and provided a major source of protein for human consumption (Hayatgheib *et al*., 2020). There has been an upsurge of disease infection due to an increased intensification in production practice leading to a 50% production loss in fish farming (Gabriel, 2019). Due to the upsurge in disease outbreaks on fish farms, antibiotics and 'traditional' chemical therapeutics are administered to minimize the economic loss associated with the disease outbreaks (Van Doan *et al*., 2019; Lieke *et al*., 2020). The use of antibiotic allows horizontal gene transfer (HGT) of antibiotic resistance genes among diverse species with the collaboration of bacterial population, which leads to drug resistant pathogens (Watts *et al*., 2017). Vaccine has also been used to mitigate against aquaculture diseases; however, it is relatively expensive, economically unwise and limited coverage for treatment of large population of fish in production units (Plant and LaPatra, 2011). Practitioners in the aquaculture industry have been trying to replace the conventional antibiotics with plant-derived supplements rather than chemical residues in aquaculture because of many factors: low cost, ready availability, fewer side effects in fish health and sustainable practice for aquatic environments (Reverter *et al*., 2014; Van Hai, 2015; Gabriel, 2019). Plant extracts have been reported to have diverse properties such as antistress, growth promotion, appetite stimulation, enhancement of tonicity, immunostimulation, aphrodisiac and anti-pathogenic effects in fish and shrimp aquaculture, these properties have been attributed to the presence of active principles such as alkaloids, terpenoids, tannins, saponins, glycosides, flavonoids, phenolics, steroids or essential oils

(Chakraborty and Hancz, 2011; Gabriel, 2019). Thus, the use of plant extracts in the aquaculture production could assist in reducing the deleterious effects of some toxicants or compounds and reduce treatment cost associated with synthetic drugs, they are also more environmentally friendly as they tend to be easily biodegradable than synthetic molecules and are less likely to produce drug resistance due to the high diversity of plant extract molecules (Olusola *et al.,* 2013). Several studies have monitored the immunological responses after intraperitoneal injection or oral administration of plant extracts in distinct fish species. The findings in the treated fish showed increased lysozyme activity, phagocytic activity, complement activity, increased respiratory burst activity and increased plasma protein concentrations (globulin and albumin) (Liu *et al.,* 2006; Wu *et al.,* 2010; Ghosh *et al.,* 2018). The effect of plant products on fish is dose-dependent and there is a potential for overdosing to occur, which necessitates determining the suitable extract concentration (Harikrishnan *et al.,* 2011a).

Acute exposure of *Clarias gariepinus* to ethanol extract of *Adenium obesum* stem bark resulted in vacuolation of hepatocytes, fatty degeneration, congestion of central vein, mononuclear cellular infiltration, hepatocyte necrosis, and degenerated hepatocytes in the liver; secondary lamellar fusion, edema and hemorrhage, epithelial detachment in the gill, proliferated and hypertrophied mucous cells, eroded epithelial surface, and thickened epidermal layer of the skin and *Adenium obesum* have been reported as a piscicide (Abalaka *et al*., 2015).

*Borreria verticillata* is a perennial shrub belonging to the family *Rubiaceae*. It is commonly called shrubby false button weed or shrubby false button wood (Burkill, 2000). It is distributed in tropical and subtropical America, Africa, Asia, and Europe (Dessein *et al.,* 2006). It originated from South and Central America (Chiquieri *et al.,* 2004). In Nigeria, *Borreria verticillata* it is common to all vegetation, particularly in Sudan savannah, where it is known by various local names.

The various species of *Borreria verticillata* are used in traditional medicine in different parts of the world, especially in Latin America, Asia, Africa and West Indies. In Brazil, the infusion of the flower extract is used as antipyretic and analgesic (Moreira *et al.,* 2010). The root extract is used as an emetic, while the leaf extract is used as antidiarrheal, and in the remedy against erysipelas and hemorrhoids (Lorenzi and Matos, 2002).

In many parts of Nigeria, as in many West African countries, the leaves are used for curative purposes as one of the mainstream traditional medicines. Studies have confirmed that the extracts of *Borreria* and *Spermacoce* species and the isolated compounds from them possessed diverse biological activities that include analgesic, anti-inflammatory, antitumor, antimicrobial, larvicidal, antioxidant, gastrointestinal, anti-ulcer, and hepatoprotective properties, with alkaloids and iridoids being the major active principles (Abdullahi *et al.,* 2014). The non-toxic effect of aqueous extract of *Borreria verticillata* in acute exposure of *Clarias gariepinus* was recently reported (Muyiwa *et al*., 2019a), which made it an ideal candidate for treatment of eco-toxicities in the aquaculture.

The aim of this study was to determine the ameliorative effects of *Borreria verticillata* aqueous extract on hematological alterations consequent to toxicity induced by dissolved *Adenium obesum* stem bark aquatic environment of *Clarias gariepinus* juveniles. With the reported chemotherapeutic effects of *Borreria verticillata* in man and rats, it is anticipated that based on this study *Borreria verticillata* could be a possible candidate to ameliorate various conditions in fish that presents the same types of pathology in the hematology profile expressed by the *Clarias gariepinus* exposure to *Adenium obesum* aqueous extract.

## MATERIALS AND METHODS

### Plant extracts

The *Adenium obesum* aqueous stem bark and *Borreria verticillata* extract were prepared as described by Muyiwa *et al*. (2019a) and (2019b), respectively.

### Fish Source and Fish Maintenance

Four hundred and fifty (450) live juvenile African sharp-tooth catfish, *C. gariepinus*, with an average weight of (21.48 ± 3.32) g and length (11.37 ± 1.23) cm, respectively, were purchased from a commercial catfish farm in Kaduna (FISHOUSE) of reputable standing. The fish were transported from Mando in Kaduna state to Ahmadu Bello University, Zaria, in two 50L plastic containers by road within the hours of 6am to 7am.The Fish were kept in a 1000L water capacity concrete tank at the Biological Science Department, Ahmadu Bello University, Zaria for acclimatization and authenticated at the Fishery Section, Department of Biological Sciences, A. B.U., Zaria, Nigeria. The fish were fed at 35% of their body weight with 2mmof Coppens Holland company feed. The acclimatization lasted for 14 days during which period fish were observed for infection and mortality.

### Experimental design

### Determination of Median Lethal Concentration ($LC_{50}$) of *Adenium obesum* and *Borreria verticillata* aqueous extract.

The median lethal dose ($LC_{50}$) of *Adenium obesum* extract of 8.3 mg/l used for this study was determined and reported by Muyiwa *et al.,* 2019a,10% of the $LC_{50}$, 0.838mg/l,was used in this study. The sub lethal doses of *Borreria verticillata* used in this study (25mg/l,50mg/l, 100mg/l,200mg/l) was based on the non-toxic nature of the extract to *Clarias gariepinus* in acute exposure as reported by Muyiwa *et al*.,2019b. The fish were allocated at random to groups, 1-10, of 15 fish per group. G1 represents the control group, 0.5 L of water was siphoned and replaced with 0.5 L un-chlorinated water on daily basis in G1, the water was refreshed completely at intervals of three days. In groups, G 2, G3, G4and G5, 25mg/l, 50mg/l, 100mg/l and 200mg/l sub lethal doses of BVE respectively were administered into the tank water, after effectively dissolving the dried BVAE in 10mls of water taken from the group tank water in order to maintain a constant concentration of the extract in the tank, G2 – G5 were

administered only the BVAE extract for 28 days. G6, were administered 0.838 mg/l of AOE only for 28 days, this represents 10% of $LC_{50}$ AOE. 10% $LC_{50}$ AOE (0.838mg/l) was administered into the water in G7– G10 followed by the administration of 25mg/l, 50mg/l, 100mg/l and 200mg/l sub lethal doses of BVAE respectively after a 6 hours' interval between administration for 28 days. Fish were fed twice daily (9am and 4pm) with 2mm coppen® company feed, extracts were administered into the fish tank after morning feeding daily for 28 days, and water was refreshed completely every three days. Blood sample were collected on day 0, 7, 14, 21 and 28.

**Collection of Blood Sample**

Exactly 1.0 ml of blood was collected from each fish via caudal veno-puncture, using the lateral approach. A 25G needle attached to 5 ml syringe was introduced through the lateral line, lateral to the body of the fish, until contact was made with the spine. An aspiration was made by gentle withdrawal to be sure the needle was in the vein and the blood was collected into a 2ml blood sample bottle containing ethylene diamine tetra acetic acid (EDTA) as anticoagulant and used for hematological evaluations.

**Hematological analysis**

The packed cell volume (PCV) was determined using standard technique as described by Rehman *et al*. (2003). Red blood cells (RBC) and total white blood cell count was determined with the Natt-Herrick solution (1:200 dilution) and the Improved Neubauer hemocytometer (Campbell and Ellis, 2007). Hemoglobin concentration (HB) was assayed colorimetrically using cyanmethemoglobin method as described by Feldmann *et al*. (2000). The differential white blood cell count was determined using the method described by Kemal (2014). Hematological indices of MCV, MCH and MCHC were calculated as described by Stockham and Scott (2002).

**Data Analysis**

Data was expressed as mean ± SEM and then subjected to Two-way Analysis of Variance (ANOVA) for statistical significance at $P < 0.05$. Tukey's multiple comparison tests for means were used to compare differences between the various means using, Statistical Package for Social Sciences (IBM SPSS) version 20.

**Ethical Statement**

Ethical clearance approval was obtained from the Ahmadu Bello University Committee on Animal Use and Care (ABUCAUC) with approval number ABUCAUC/2017/014 for this study.

**RESULTS**

**Mean Red Blood Cell Count Changes Following Fish Exposure to Sublethal Concentration of *Adenium obesum* and treatment with different concentrations of *Borreria verticillata* aqueous extract**

The differences between the mean RBC counts in the different groups on day 0 were not significant ($P > 0.05$). Also, the mean RBC count in groups 2-5 did not vary significantly ($P > 0.05$) when compared with the corresponding values in G1, throughout the 28-day experimental period. A decrease in the mean RBC count was observed in G6, beginning from day 7 post exposure to 10% $LC_{50}$ AOE ($2.72 \pm 0.01$ x$10^{12}$/L) to reach a significantly ($P < 0.05$) lower value ($2.00 \pm 0.11$ x$10^{12}$/L) at termination of the experiment on day 28 (Table 1). The mean RBC counts in groups 7-10, exposed to *Adenium obesum* and treated with different concentrations of *Borreria verticillata,* showed similar decrease but their values were significantly higher than G6. The individual values of RBC count of the groups, G7, G8, G9 and G10 at termination of the experiment was however significantly ($P < 0.05$) higher than that of group 6.

**Mean Hemoglobin (Hb) Concentration Changes Following Fish Exposure to Sublethal Concentration of *Adenium obesum* and treatment with different concentrations of *Borreria verticillata* aqueous extract**

The mean hemoglobin concentration significantly ($P < 0.05$) decreased from 11.23±0.12 g/dl on day 0 to 10.72 ± 0.01g/dl on day 28 in G6 compared to G1-G5 (groups exposed to BVAE alone) where the values remain non significantly ($P > 0.05$) unchanged. Groups, G7-G10, treated with different concentrations of *Borreria verticillata* aqueous extract had significant ($P < 0.05$) increase in the mean hemoglobin concentration on days 7, 14 and 28, during the study (Table 3).

**Mean Packed Cell Volume (PVC) Changes Following Fish Exposure to Sublethal Concentration of *Adenium obesum* and treatment with different concentrations of *Borreria verticillata* aqueous extract**

Following exposure to 10% $LC_{50}$ AOE, there was non-significant ($P>0.05$) decrease in mean PCV values, from 30.97±0.01% (day 7) to 29.37±0.27% (day 28) in G6. The mean values of PCV obtained for groups, G2-G5 insignificantly ($P>0.05$) decreased during the 28-days period compared to group 1(control). However, it was observed that the mean PCV values in groups, G7-G10 varied significantly ($P<0.05$) on day 14 and insignificantly ($P > 0.05$) increased from days 21 to 28 of the study, compared to the insignificant (P>0.05) decrease in G6 (Table 3).

**Mean Corpuscular Volume Changes Following Fish Exposure to Sub Lethal Concentration of *Adenium obesum* and treatment with different concentrations of *Borreria verticillata* aqueous extract**

The difference in the mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values between the groups on day 0 and day 7 were not significant ($P>0.05$). The mean corpuscular volume (Table 5), mean corpuscular hemoglobin (Table 6) and mean corpuscular hemoglobin concentration (Table 7) all had a similar pattern of significant ($P<0.05$) decrease from days 7 to 28 in G6, the group exposed to 10% $LC_{50}$ AOE for 28 days. Mean values of the erythrocytic indices insignificantly ($P>0.05$) increased in the treatment groups G7-G10, from days 7 – 28, where the fish were treated with BVAE after exposure to 10% $LC_{50}$ AOE.

**The Mean Total White Blood Cell Changes Following Fish Exposure to Sublethal Concentration of *Adenium obesum* and treatment with different concentrations of *Borreria verticillata* aqueous extract**

The total white blood cell count of the juvenile *C. gariepinus* fish in G6, insignificantly ($P$>0.05) increased from 17.98±0.04x$10^9$ cells/L on day 7 to 18.80±0.06 x $10^9$ cells/L on day 28. These values were higher than those of groups, G1-G5 and G7-G10. However, there was a concentration (BVAE) and time dependent decrease in the total white blood cell counts in the BVAE treated groups, G7-G10 (Table 8). Similarly, there were significant ($P$<0.05) increase in differential counts of heterophils (Table 9), eosinophils (Table 10), monocytes (Table 11) and lymphocytes (Table 12) in G6. For all the white blood cell types herein evaluated, the increase was first observed on day 7, except for monocyte counts, the increase of which was delayed until day 14. The mean values of differential white blood cell count also decreased significantly ($P$<0.05) with increase in the concentration of BVAE in the treatment groups, G7-G10 compared to G6, the group exposed to 10% $LC_{50}$ AOE only'.

**Table 1:** Mean Red Blood Cell Count (RBC) of *Clarias gariepinus* exposed to AOE and treated with BVAE

| DAYS | DAY 0 | DAY 7 | DAY 14 | DAY 21 | DAY 28 |
|---|---|---|---|---|---|
| GROUPS | RED BLOOD CEL COUNT L (x$10^{12}$/L) | | | | |
| G1 | 2.69 ± 0.01$^a$ | 2.71 ± 0.01$^a$ | 2.73 ± 0.02$^a$ | 2.73 ± 0.03$^a$ | 2.73 ± 0.02$^a$ |
| G2 | 2.68 ± 0.01$^a$ | 2.70 ± 0.02$^a$ | 2.70 ± 0.03$^a$ | 2.71 ± 0.03$^a$ | 2.73 ± 0.03$^a$ |
| G3 | 2.67 ± 0.01$^a$ | 2.69 ± 0.01$^a$ | 2.71 ± 0.01$^a$ | 2.74 ± 0.03$^a$ | 2.75 ± 0.04$^a$ |
| G4 | 2.71 ± 0.02$^a$ | 2.72 ± 0.01$^a$ | 2.70 ± 0.01$^a$ | 2.71 ± 0.02$^a$ | 2.70 ± 0.01$^a$ |
| G5 | 2.73 ± 0.03$^a$ | 2.70 ± 0.02$^a$ | 2.71 ± 0.02$^a$ | 2.70 ± 0.01$^a$ | 2.70 ± 0.05$^a$ |
| G6 | 2.72 ± 0.01$^a$ | 2.56 ± 0.01$^b$ | 2.30 ± 0.01$^b$ | 2.15 ± 0.03$^b$ | 2.00 ± 0.11$^b$ |
| G7 | 2.72 ± 0.01$^a$ | 2.57 ± 0.01$^b$ | 2.37 ± 0.01$^c$ | 2.22 ± 0.01$^c$ | 2.13 ± 0.01$^c$ |
| G8 | 2.70 ± 0.01$^a$ | 2.59 ± 0.01$^b$ | 2.46 ± 0.01$^d$ | 2.38 ± 0.01$^d$ | 2.29 ± 0.01$^d$ |
| G9 | 2.70 ± 0.01$^a$ | 2.60 ± 0.02$^b$ | 2.51 ± 0.01$^e$ | 2.52 ± 0.01$^e$ | 2.49 ± 0.01$^e$ |
| G10 | 2.69 ± 0.01$^a$ | 2.65 ± 0.01$^c$ | 2.69 ± 0.01$^{ff}$ | 2.63 ± 0.02$^f$ | 2.68 ± 0.01$^f$ |

All values indicated by different letters are significantly ($P$<0.05) different between the groups compared to the control. G1 = Control, G2 = 25 mg/l BAVE; G3 = 50 mg/l BVAE; G4 = 100 mg/l BVE;G5 = 200 mg/l BVAE;G6 = 10% $LC_{50}$ AOE; G7 = G2+G6;G8 =G3+G6;G9=G4+G6;G10=G5+G6.,Keys: AOE= *Adenium obesum* aqueous extract; BVAE= *Borreria verticillata* aqueous extract.

**Table 2:** Mean Hemoglobin Concentration (Hb) of *Clarias gariepinus* exposed to AOE and treated with BVAE

| DAYS | DAY 0 | DAY 7 | DAY 14 | DAY 21 | DAY 28 |
|---|---|---|---|---|---|
| GROUPS | HEMOGLOBIN CONCENTRATION (g/dl) | | | | |
| G1 | 11.36 ± 0.03 | 11.38 ± 0.03$^a$ | 11.39 ± 0.03$^a$ | 11.40 ± 0.03$^a$ | 11.41 ± 0.04$^a$ |
| G2 | 11.35 ± 0.03 | 11.37 ± 0.03$^a$ | 11.38 ± 0.03$^a$ | 11.40 ± 0.04 $^a$ | 11.41 ± 0.03$^a$ |
| G3 | 11.34 ± 0.04 | 11.35 ± 0.04$^b$ | 11.36 ± 0.04 | 11.37 ± 0.04 | 11.38 ± 0.04 |
| G4 | 11.38 ± 0.03 | 11.39 ± 0.02$^a$ | 11.40 ± 0.02 | 11.41 ±0.02$^a$ | 11.42 ± 0.02$^a$ |
| G5 | 11.36 ± 0.03 | 11.37 ± 0.04$^a$ | 11.37 ± 0.04$^a$ | 11.38 ± 0.04 | 11.39 ± 0.04 |
| G6 | 11.23 ± 0.12 | 11.00 ± 0.01$^c$ | 10.85 ± 0.01$^b$ | 10.72 ± 0.01$^b$ | 10.65 ± 0.01$^b$ |
| G7 | 11.34 ± 0.03 | 11.05 ± 0.01$^d$ | 11.00 ± 0.02$^c$ | 11.07 ± 0.01$^c$ | 11.17 ± 0.03$^c$ |
| G8 | 11.35 ± 0.05 | 11.10 ± 0.03$^e$ | 11.10 ± 0.01$^d$ | 11.08 ± 0.01$^d$ | 11.23 ± 0.01$^d$ |
| G9 | 11.29 ± 0.01 | 11.14 ± 0.01$^f$ | 11.20 ± 0.01$^e$ | 11.09 ± 0.01$^e$ | 11.30 ± 0.01$^e$ |
| G10 | 11.31± 0.01 | 11.20 ± 0.01$^g$ | 11.30 ± 0.01$^f$ | 11.12 ± 0.01$^f$ | 11.38 ± 0.01$^f$ |

All values indicated by different letters are significantly (P<0.05) different between the groups compared to the control. G1 = Control; G2 = 25mg/l BVE;G3 = 50mg/l BVE; G4 = 100mg/l BVE; G5= 200mg/l BVE; G6 = 10% LC50 AOE; G7 = G2+G6; G8 = G3+G6; G9=G4+G6;G10=G5+G6.,Keys: AOE= *Adenium obesum* aqueous extract; BVAE= *Borreria verticillata* aqueous extract.

**Table 3:** Mean Packed Cell Volume (PCV) of *Clarias gariepinus* juveniles exposed to AOE and treated with BVAE

| DAYS | DAY 0 | DAY 7 | DAY 14 | DAY 21 | DAY 28 |
|---|---|---|---|---|---|
| GROUPS | PACKED CELL VOLUME (%) | | | | |
| G1 | 32.35 ± 1.66$^a$ | 32.36 ± 0.66$^a$ | 32.71 ± 0.33$^a$ | 32.72 ± 0.33$^a$ | 32.73 ± 0.33$^a$ |
| G2 | 36.35 ± 3.01$^a$ | 32.37 ± 0.66$^a$ | 32.72 ± 0.33 | 33.08 ± 0.01$^a$ | 33.09 ± 0.01$^a$ |
| G3 | 32.34 ± 0.67$^a$ | 32.36 ± 0.67$^a$ | 32.71 ± 0.34 | 33.05 ± 0.01$^a$ | 33.06 ± 0.01$^a$ |
| G4 | 32.40 ± 0.67$^a$ | 32.41 ± 0.66$^a$ | 32.42 ± 0.66 | 32.76 ± 0.33$^a$ | 32.77 ± 0.33$^a$ |
| G5 | 32.42 ± 0.67$^a$ | 32.42 ± 0.67$^a$ | 32.43 ± 0.67 | 32.44 ± 0.67$^a$ | 32.47 ± 0.67$^a$ |
| G6 | 32.36 ± 0.67$^a$ | 30.97 ±0.01$^b$ | 29.93 ±0.33$^b$ | 29.60±0.20$^b$ | 29.37 ± 0.27$^b$ |
| G7 | 32.37±0.67$^a$ | 31.04±0.01$^b$ | 30.04±0.01$^b$ | 30.20±0.06$^c$ | 30.94±0.01$^a$ |
| G8 | 32.34±0.66$^a$ | 31.14±0.02$^b$ | 31.47±0.03$^b$ | 30.72±0.01$^c$ | 31.27±0.12$^a$ |
| G9 | 32.38±0.67$^a$ | 31.19±0.03$^b$ | 31.73±0.03$^b$ | 30.94±0.01$^c$ | 31.83±0.03$^a$ |
| G10 | 33.00±0.01$^a$ | 31.31±0.01$^b$ | 32.00±0.01$^b$ | 32.00±0.01$^a$ | 32.20±0.05$^a$ |

All values indicated by different letters are significantly (P<0.05) different between the groups compared to the control. G1 = Control; G2 = 25mg/l BVE; G3 = 50mg/l BVE; G4= 100mg/l BVE; G5= 200mg/l BVE; G6 = 10% LC50 AOE; G7 = G2+G6; G8 = G3+G4; G9=G4+G6; G10=G5+G6. Keys: AOE= *Adenium obesum* aqueous extract; BVAE= *Borreria verticillata* aqueous extract.

**Table 4:** Mean Corpuscular Volume (MCV) of *Clarias gariepinus* juveniles exposed to AOE and treated with BVAE

| DAYS | DAY 0 | DAY 7 | DAY 14 | DAY 14 | DAY 21 |
|---|---|---|---|---|---|
| GROUPS | MEAN CORUSCULAR VOLUME (%) | | | | DAY 28 |
| G1 | 134.97±0.12[a] | 135.31±0.47[a] | 135.64±0.79[a] | 135.08±0.12[a] | 135.10±0.25[a] |
| G2 | 135.62±0.61[a] | 134.98±0.14a | 134.96±0.15[a] | 134.97±0.13[a] | 135.02±0.15[a] |
| G3 | 135.01±0.16[a] | 134.96±0.13a | 134.96±0.16[a] | 134.98±0.13[a] | 135.09±0.21[a] |
| G4 | 135.00±0.15[a] | 134.98±0.14a | 134.96±0.09[a] | 134.96±0.13[a] | 134.98±0.14[a] |
| G5 | 134.96±0.12[a] | 134.98±0.15a | 134.96±0.15[a] | 134.96±0.13[a] | 134.94±0.15[a] |
| G6 | 134.99±0.16[a] | 133.31±0.01[b] | 130.23±0.01[b] | 130.02±0.13[b] | 128.79±0.01[b] |
| G7 | 135.03±0.19[a] | 133.35±0.01[b] | 131.35±0.01[b] | 132.35±0.02[c] | 130.35±0.01[c] |
| G8 | 135.00±0.16[a] | 133.38±0.01[b] | 132.48±0.01[c] | 132.48±0.01[c] | 131.48±0.01[c] |
| G9 | 135.03±0.19[a] | 133.39±0.01[b] | 133.54±0.02[d] | 132.58±0.01[c] | 131.68±0.01[c] |
| G10 | 134.99±0.16[a] | 133.41±0.01 | 133.66±0.01[e] | 133.60±0.01[c] | 131.77±0.01[c] |

All values indicated by different letters are significantly ($P$<0.05) different between the groups compared to the control.1, 2,3,4, 5, 6, 7,8,9 and 10; G1 = Control; G2 = 25mg/l BVE; G3 = 50mg/l BVE; G4 = 100mg/l BVE; G5= 200mg/l BVE; G6 = 10% LC50 AOE; G7 = G2+G6; G8 = G3+G6; G9=G4+G6; G10=G5+G6. Keys: AOE= *Adenium obesum* aqueous extract; BVAE= *Borreria verticillata* aqueous extract.

**Table 5:** Mean Corpuscular Hemoglobin (MCH) of *Clarias gariepinus* juveniles exposed to AOE and treated with BVAE

| DAYS | DAY 0 | DAY 7 | DAY 14 | DAY 21 | DAY 28 |
|---|---|---|---|---|---|
| GROUPS | MEAN CORPUSCULAR HEMOGLOBIN (pg) | | | | |
| G1 | 40.29±0.48[a] | 40.30±0.48[a] | 40.31±0.48[a] | 40.31±0.48[a] | 40.33±0.48[a] |
| G2 | 40.32±0.48[a] | 40.32±0.50[a] | 40.32±0.50[a] | 40.33±0.50[a] | 40.34±0.48[a] |
| G3 | 40.32±0.49[a] | 40.31±0.49[a] | 40.32±0.48[a] | 40.31±0.50[a] | 40.31±0.48[a] |
| G4 | 40.28±0.49[a] | 40.29±0.49[a] | 40.30±0.49[a] | 40.31±0.49[a] | 40.32±0.48[a] |
| G5 | 40.30±0.47[a] | 40.30±0.48[a] | 40.31±0.48[a] | 40.32±0.48[a] | 40.33±0.48[a] |
| G6 | 40.30±0.46[a] | 37.47±0.11[b] | 36.50±0.05[b] | 35.57±0.12[b] | 34.67±0.14[b] |
| G7 | 40.32±0.47[a] | 37.37±0.25[b] | 37.01±0.01[b] | 37.04±0.01[c] | 37.37±0.25[c] |
| G8 | 40.28±0.50[a] | 38.64±0.30[b] | 38.01±0.01[b] | 38.64±0.30[c] | 38.02±0.01[d] |
| G9 | 40.28±0.49[a] | 39.00±0.01[b] | 39.04±0.01[b] | 39.00±0.01[d] | 39.00±0.01[e] |
| G10 | 40.29±0.48[a] | 40.52±0.06[a] | 39.95±0.02[a] | 39.95±0.02[d] | 39.95±0.02[e] |

All values indicated by different letters are significantly (P<0.05) different between the groups compared to the control. G1= Control; G2 = 25mg/l BVE; G3 = 50mg/l BVE; G4 = 100mg/l BVE; G5= 200mg/l BVE; G6 = 10% LC50 AOE; G7 = G2+G6; G8 = G3+G6 G8=G4+G6; G10=G5+G6. Keys: AOE= *Adenium obesum* aqueous extract; BVAE= *Borreria verticillata* aqueous extract.

**Table 6:** Mean Corpuscular Hemoglobin Concentration (MCHC) of *Clarias gariepinus* juveniles exposed to AOE and treated with BVAE

| DAYS | DAY 0 | DAY 7 | DAY 14 | DAY 21 | DAY 28 |
|---|---|---|---|---|---|
| GROUPS | MEAN CORPUSCULAR HEMOGLOBIN CONCENTRATION (%) | | | | |
| G1 | 32.63±0.33[a] | 33.28±0.57[a] | 33.29±0.57[a] | 33.30±0.57[a] | 33.31±0.57[a] |
| G2 | 32.27±0.58[a] | 33.27±0.57[a] | 33.25±0.56[a] | 33.25±0.58[b] | 33.24±0.56[b] |
| G3 | 31.93±0.89[a] | 33.28±0.57[a] | 33.01±0.58[a] | 33.24±0.58[b] | 33.27±0.57[c] |
| G4 | 33.26±0.57[a] | 33.27±0.57[a] | 33.04±0.57[a] | 33.25±0.57[b] | 33.28±0.57[c] |
| G5 | 33.27±0.57[a] | 33.25±0.59[a] | 33.03±0.03[a] | 33.20±0.59[c] | 33.27±0.57[c] |
| G6 | 33.29±0.57[a] | 27.47±0.33[b] | 26.47±0.03[b] | 25.57±0.28[d] | 24.26±0.01[d] |
| G7 | 33.28±0.57[a] | 28.24±0.01[c] | 29.00±0.01[c] | 29.00±0.01[e] | 29.07±0.06[e] |
| G8 | 33.29±0.57[a] | 29.29±0.01[d] | 29.29±0.01[d] | 29.20±0.01[f] | 29.24±0.02[f] |
| G9 | 32.17±0.58[a] | 29.34±0.03[e] | 29.34±0.01[e] | 29.74±0.02[e] | 30.57±0.03[g] |
| G10 | 32.26±0.57[a] | 30.26±0.13[f] | 30.89±0.09[f] | 31.12±0.13[f] | 31.39±0.02[h] |

All values indicated by different letters are significantly (P<0.05) different between the groups compared to the control. G1 = Control; G2 = 25mg/l BVE; G3 = 50mg/l BVE; G4= 100mg/l BVE; G5= 200mg/l BVE; G6= 10% LC50 AOE; G7 =G2+G6; G8 =G3+G6; G9=G4+G6; G10=G5+G6. Keys: AOE= *Adenium obesum* aqueous extract; BVAE= *Borreria verticillata* aqueous extract.

## DISCUSSION

Blood parameters profile reveals the patho-physiological state of the whole body, the evaluation of hematological profile in animals has been used as a means of diagnosing disease conditions and as early warning signs of environmental contamination (Adhari and Sarkar,2014; Abdel-Tawwab *et al*., 2019; Qyli *et al*., 2020). In this study, there was a significant decrease ($p$ < 0.05) in the RBC, Hb, PVC, MCV, MCH and MCHC hematological parameters of *C. gariepinus* in G6, where they were exposed to 5% $LC_{50}$ AOE alone for 28 days. A reduction in RBC, PCV and Hb values was reported in *Clarias gariepinus* exposed to *Telfairia occidentalis* extract (Agwu *et al*., 2016). A decrease in the values of RBC, Hb, PVC, MCV, MCH and MCHC was reported by Abalaka *et al*. (2014), in wistar rats exposed to *Adenium obesum* ethanol extract administered through feed. The mean RBC count, PCV, HB concentration and TWBC in Groups, G2 (25mg/l BVAE), G3(50mg/l BVAE), G4 (100mg/l BVAE) and G5 (200mg/l BVAE) respectively did not show any significant variations throughout the

experimental period and were similar to the mean value of the control, this implied that the doses of the *Borreria verticillata* aqueous extract did not cause any hematological alterations in the fish, the non-toxic effect of *Borreria verticillata* aqueous extract even at a dose value >1000mg/l of the extract has been reported by Muyiwa *et al.*(2019),this most have been responsible for the non hematoxicity observed in the hematological profile in the groups where BVAE was administered alone for the 28 days.

**Table 7:** Mean Total White Blood Cell of *Clarias gariepinus* exposed to AOE and treated with BVAE

| DAYS | DAY 0 | DAY 7 | DAY 14 | DAY 21 | DAY 28 |
|---|---|---|---|---|---|
| GROUPS | TOTAL WHITE BLOOD CELL COUNT ($x10^9$/L) | | | | |
| G1 | 17.96±0.03$^{a}$ | 17.99±0.01$^{a}$ | 18.00±0.02$^{a}$ | 17.99±0.03$^{a}$ | 18.00±0.04$^{a}$ |
| G2 | 17.96±0.04$^{a}$ | 17.98±0.02 | 17.99±0.03 | 18.00±0.03 | 18.01±0.03 |
| G3 | 17.93±0.07$^{a}$ | 17.96±0.04 | 17.97±0.06 | 17.98±0.03 | 17.98±0.06 |
| G4 | 17.90±0.11$^{a}$ | 18.00±0.02 | 18.01±0.03 | 18.02±0.03 | 18.02±0.04 |
| G5 | 17.87±0.14$^{a}$ | 18.00±0.02 | 17.98±0.06 | 17.92±0.04 | 17.92±0.13 |
| G6 | 17.98±0.04$^{a}$ | 18.14±0.01$^{b}$ | 18.22±0.01$^{b}$ | 18.50±0.06$^{b}$ | 18.80±0.06$^{b}$ |
| G7 | 17.98±0.05$^{a}$ | 18.10±0.01$^{c}$ | 18.16±0.01$^{cj}$ | 18.27±0.01$^{c}$ | 18.37±0.01$^{c}$ |
| G8 | 17.98±0.06$^{a}$ | 18.07±0.01$^{d}$ | 18.12±0.01$^{d}$ | 18.22±0.01$^{d}$ | 18.32±0.01$^{d}$ |
| G9 | 17.98±0.06$^{a}$ | 18.08±0.01$^{d}$ | 18.10±0.01$^{e}$ | 18.19±0.01$^{e}$ | 18.26±0.01$^{e}$ |
| G10 | 17.98±0.07$^{a}$ | 18.04±0.01$^{e}$ | 18.08±0.07$^{f}$ | 18.13±0.01$^{fg}$ | 18.09±0.01$^{f}$ |

All values indicated by different letters are significantly (P<0.05) different between the groups when compared to the control. G1 = Control; G2 = 25mg/l BVE; G3 = 50mg/l BVE; G4 = 100mg/l BVE; G5 = 200mg/l BVE; G6 = 10% LC50 AOE; G7 = G2+G3; G8 = G3+G4; G9=G4+G6; G10=G5+G6. Keys: AOE=*Adenium obesum* aqueous extractstem bark; BVE= Aqueous extract Borreria verticillata aerial part.

**Table 8:** Mean Heterophil Count of *Clarias gariepinus* exposed to AOE and treated with BVAE

| DAYS | DAY 0 | DAY 7 | DAY 14 | DAY 21 | DAY 28 |
|---|---|---|---|---|---|
| GROUPS | HETEROPHIL COUNT ($x10^9$/L) | | | | |
| G1 | 0.93±0.03$^{a}$ | 0.93±0.03$^{a}$ | 0.93±0.03$^{a}$ | 0.93±0.03$^{a}$ | 0.94±0.03$^{a}$ |
| G2 | 0.93±0.03$^{a}$ | 0.93±0.02$^{a}$ | 0.93±0.02$^{a}$ | 0.93±0.02$^{a}$ | 0.94±0.02$^{a}$ |
| G3 | 0.93±0.04$^{a}$ | 0.93±0.04$^{a}$ | 0.93±0.03$^{a}$ | 0.93±0.04$^{a}$ | 0.94±0.04$^{a}$ |
| G4 | 0.93±0.04$^{a}$ | 0.93±0.03$^{a}$ | 0.93±0.02$^{a}$ | 0.93±0.03$^{a}$ | 0.93±0.02$^{a}$ |
| G5 | 0.92±0.03$^{a}$ | 0.93±0.03$^{a}$ | 0.93±0.03$^{a}$ | 0.93±0.05$^{a}$ | 0.94±0.03$^{a}$ |
| G6 | 0.93±0.02$^{a}$ | 1.02±0.07$^{b}$ | 1.05±0.04$^{b}$ | 1.09±0.01$^{b}$ | 1.12±0.02$^{b}$ |
| G7 | 0.93±0.03$^{a}$ | 1.00±0.01$^{b}$ | 1.01±0.00$^{b}$ | 1.02±0.01$^{c}$ | 1.07±0.01$^{c}$ |
| G8 | 0.93±0.02$^{a}$ | 0.98±0.02$^{b}$ | 0.96±0.03$^{c}$ | 0.98±0.03$^{d}$ | 1.04±0.03$^{dij}$ |
| G9 | 0.93±0.01$^{a}$ | 0.96±0.03$^{b}$ | 0.94±0.02$^{d}$ | 0.96±0.01$^{e}$ | 1.00±0.03$^{e}$ |
| G10 | 0.93±0.01$^{a}$ | 0.94±0.01$^{c}$ | 0.92±0.01$^{a}$ | 0.91±0.01$^{f}$ | 0.92±0.01$^{f}$ |

All values indicated by different letters aresignificantly (*P*<0.05) different between the groups when compared to the control, G1= Control; G2= 25mg/l BVE; G3 = 50mg/l BVE; G4 = 100mg/l BVE; G5 = 200mg/l BVE; G6 = 10% LC50 AOE; G7 = G2+G6; G8 = G3+G6; G9=G4+G6; G10=G5+G6. Keys: AOE= *Adenium obesum* aqueous extract; BVAE= *Borreria verticillata* aqueous extract.

**Table 9:** Mean Eosinophil Count of *Clarias gariepinus* exposed to AOE and treated with BVAE

| DAYS | DAY 0 | DAY 7 | DAY 14 | DAY 21 | DAY 28 |
|---|---|---|---|---|---|
| GROUPS | EOSINOPHIL COUNT ($x10^9$/L) | | | | |
| G1 | 0.37±0.03$^{a}$ | 0.38±0.04$^{a}$ | 0.38±0.04$^{a}$ | 0.38±0.04$^{a}$ | 0.37±0.01$^{afgh}$ |
| G2 | 0.37±0.03$^{a}$ | 0.37±0.03$^{a}$ | 0.37±0.03$^{a}$ | 0.37±0.03$^{a}$ | 0.37±0.03$^{a}$ |
| G3 | 0.37±0.02$^{a}$ | 0.37±0.02$^{a}$ | 0.37±0.03$^{a}$ | 0.38±0.03$^{a}$ | 0.38±0.02$^{a}$ |
| G4 | 0.36±0.03$^{a}$ | 0.37±0.03$^{a}$ | 0.37±0.03$^{a}$ | 0.37±0.03$^{a}$ | 0.37±0.03$^{a}$ |
| G5 | 0.37±0.02$^{a}$ | 0.37±0.02$^{a}$ | 0.38±0.02$^{a}$ | 0.38±0.02$^{a}$ | 0.38±0.01$^{a}$ |
| G6 | 0.37±0.02$^{a}$ | 0.48±0.03$^{b}$ | 0.50±0.02$^{b}$ | 0.52±0.02$^{b}$ | 0.56±0.04b |
| G7 | 0.37±0.03$^{a}$ | 0.43±0.03$^{c}$ | 0.44±0.03$^{c}$ | 0.45±0.03$^{c}$ | 0.46±0.03$^{cfij}$ |
| G8 | 0.37±0.02$^{a}$ | 0.41±0.03$^{d}$ | 0.42±0.03$^{d}$ | 0.43±0.03$^{d}$ | 0.44±0.03$^{dfj}$ |
| G9 | 0.37±0.01$^{a}$ | 0.39±0.03$^{e}$ | 0.40±0.03$^{e}$ | 0.41±0.03$^{e}$ | 0.42±0.01 |
| G10 | 0.38±0.01$^{a}$ | 0.37±0.03$^{f}$ | 0.38±0.03$^{f}$ | 0.39±0.03$^{f}$ | 0.39±0.03f$^{fgh}$ |

All values indicated by different letters are significantly (*P*<0.05) different between the groups when compared to the control. G10 G1 = Control; G2 = 25mg/l BVAE; G3 = 50mg/l BVAE; G4 = 100mg/l BVAE; G5 = 200mg/l BVAE; G6 = 10% LC50 AOE; G7 = G2+G6; G8 = G3+G6; G9=G4+G6; G10=G5+G6. Keys: AOE= *Adenium obesum* aqueous extract; BVAE= *Borreria verticillata* aqueous extract.

The decreased mean values of RBC, PCV, Hb, MCV, MCH, MCHC and TWBC, observed in the group 6 , exposed to the aqueous extract of *Adenium obesum* stem bark, when compared to the corresponding values of Groups 7-10 (groups exposed to AOE and treated with BVAE ) and Groups 2-5 (groups exposed to different concentrations of BVAE only) were in contrast with the report of Abalaka *et al*. (2015), who reported significantly high mean values in the hematological profile of wistar rats exposed to *Adenium obesum* extract through feed at 300mg/kg.b.wt, 2000mg/kg.b.wt and 5000mg/kg.b.wt respectively. A decrease in mean RBC, PCV and Hb consequent to plant toxicity is not only unique to *Adenium obesum* extract, Agwu *et al.* (2018) had reported similar

findings following exposure of *Clarias gariepinus* fingerlings to root extract of *Telfairia occidentalis*. Also, white tilapia, *Oreochromis niloticus,* had also shown a decrease in the values of RBC, Hb, and PCV on being exposed to almond (*Terminalia catappa),* pawpaw (*Carica papaya),* Neem (*Azadirachta indica),* Tobacco (*Nicotiana tobacum)* and Cassava (*Manihot esculenta*) extracts (Fafioye, 2012). Many other investigators reported decrease of RBC, Hb, PCV and erythrocytic indices due to extracts with toxic constituents (Olusegun and Adebayo, 2014; Okwogu *et al.,* 2015; Nwani *et al.,* 2016; Muyiwa *et al*.,2020; Camara *et al.*, 2022). The exact mechanism of toxicity due to extract of *Adenium obesum* and leading to significant decreases in mean PCV, Hb and RBC count was not investigated in this study , but, *Adenium obesum* has been reported to cause hematotoxicity through direct chemical injury from its cardiac glycosides and other toxic secondary metabolites, with loss or destruction of RBC and the mechanisms are reasonably well-described from toxicology studies in fish and mammals (Payne,2005; Ahmed *et al*.,2017).The decrease in RBC, HB and PCV levels may be attributed to the malfunctioning of the erythropoietic tissue due to the hemolyzing and shrinkage effects of AOE, or hemodilution which might have resulted from the osmoregulatory dysfunction of the gill. These findings concur with the previous observations of Velisek *et al*. (2010) and Marzouk *et al*. (2012), who reported a marked decrease in RBC, HB and PCV in *Cyprinus carpio* and *Clarias gariepinus* exposed to terbutryn and atrazine, respectively. Hamed and El-Sayed (2018) also recorded similar observations in *Oreochromis niloticus* treated with pendimethalin herbicide. The AOE-induced decrease in MCV, MCH and MCHC may be due to direct responses of erythrocyte membranes to structural damage resulting in hemolysis and impairment in synthesis of haemoglobin or stress related release of erythrocytes from the spleen and hypoxia (Mekkawy *et al*., 2013).

**Table 10:** Mean Monocyte Count of *Clarias gariepinus* exposed to AOE and treated with BVAE

| DAYS | DAY 0 | DAY 7 | DAY 14 | DAY 21 | DAY 28 |
|---|---|---|---|---|---|
| GROUPS | MONOCYTE COUNT ($x10^9$/L) | | | | |
| G1 | 2.54±0.03 $^{a}$ | 2.55±0.02$^{a}$ | 2.55±0.03$^{a}$ | 2.55±0.01$^{a}$f | 2.55±0.01$^{a}$ |
| G2 | 2.53±0.01$^{a}$ | 2.54±0.01$^{a}$ | 2.54±0.01$^{a}$ | 2.54±0.01$^{a}$ | 2.54±0.02$^{a}$ |
| G3 | 2.52±0.01$^{a}$ | 2.53±0.02$^{a}$ | 2.53±0.02$^{a}$ | 2.54±0.02$^{a}$ | 2.54±0.02$^{a}$ |
| G4 | 2.53±0.01$^{a}$ | 2.54±0.01$^{a}$ | 2.55±0.01$^{a}$ | 2.55±0.01$^{a}$ | 2.55±0.01$^{a}$ |
| G5 | 2.53±0.03$^{a}$ | 2.54±0.01$^{a}$ | 2.54±0.02$^{a}$ | 2.53±0.01$^{a}$ | 2.54±0.01$^{a}$ |
| G6 | 2.54±0.03$^{a}$ | 2.58±0.02$^{b}$ | 2.65±0.01$^{b}$ | 2.79±0.34$^{b}$ | 2.94±0.33$^{b}$ |
| G7 | 2.55±0.06 $^{a}$ | 2.57±0.01$^{c}$ | 2.60±0.01$^{c}$ | 2.68±0.01$^{c}$ | 2.70±0.01$^{c}$ |
| G8 | 2.55±0.05$^{a}$ | 2.56±0.01$^{d}$ | 2.58±0.02$^{d}$ | 2.65±0.02$^{d}$ | 2.67±0.01$^{d}$ |
| G9 | 2.55±0.07$^{a}$ | 2.55±0.01$^{a}$ | 2.58±0.01$^{d}$ | 2.63±0.01$^{e}$ | 2.64±0.02$^{e}$ |
| G10 | 2.55±0.06$^{a}$ | 2.55±0.01$^{a}$ | 2.56±0.01$^{e}$ | 2.60±0.01$^{f}$ | 2.60 ±0.01$^{f}$ |

All values indicated by different letters are significantly ($P$<0.05) different between the groups when compared to the control, G1.; G1 = Control; G2 = 25mg/l BVAE; G3 = 50mg/l BVAE; G4 = 100mg/l BVAE; G5= 200mg/l BVAE; G6= 10% $LC_{50}$ AOE; G7 = G2+G6; G8 = G3+G6; G9=G4+G6; G10=G5+G6. Keys: AOE= *Adenium obesum* aqueous extract; BVAE= *Borreria verticillata* aqueous extract.

**Table 11:** Mean Lymphocyte Count of *Clarias gariepinus* exposed to AOE and treated with BVAE

| DAYS | DAY 0 | DAY 7 | DAY 14 | DAY 21 | DAY 28 |
|---|---|---|---|---|---|
| GROUPS | LYMPHOCYTE COUNT ($x10^9$/L) | | | | |
| G1 | 13.24±2.18$^{a}$ | 13.44±1.66$^{a}$ | 13.45±1.66$^{a}$ | 13.44±1.66$^{a}$ | 13.45±1.66$^{a}$ |
| G2 | 13.42±1.66$^{a}$ | 13.43±1.66 | 13.45±1.66 | 13.45±1.66 | 13.46±1.66 |
| G3 | 13.34±1.33$^{a}$ | 13.36±1.32 | 13.37±1.34 | 13.38±1.34 | 13.38±1.34 |
| G4 | 13.38±1.65$^{a}$ | 13.45±1.65 | 13.46±1.65 | 13.46±1.65 | 13.46±1.66 |
| G5 | 13.30±1.32$^{a}$ | 13.39±1.34 | 13.37±1.33 | 13.33±1.33 | 13.33±1.33 |
| G6 | 13.44±1.65$^{a}$ | 14.26±0.01$^{b}$ | 14.51±0.01$^{b}$ | 15.18±0.02$^{b}$ | 15.41±0.02$^{b}$ |
| G7 | 13.43±1.68$^{a}$ | 14.17±0.01$^{c}$ | 14.23±0.01$^{c}$ | 14.51±0.01$^{c}$ | 14.78±0.01$^{c}$ |
| G8 | 13.38±1.84$^{a}$ | 13.95±0.01$^{d}$ | 14.00±0.01$^{d}$ | 13.90±0.01$^{d}$ | 14.54±0.01$^{d}$ |
| G9 | 13.38±1.88$^{a}$ | 13.46±1.33$^{e}$ | 13.60±0.01$^{e}$ | 13.68±0.01$^{e}$ | 14.29±0.01$^{e}$ |
| G10 | 13.38±1.83$^{a}$ | 13.18±0.01$^{fghi}$ | 13.39±0.01$^{f}$ | 13.38±0.01$^{f}$ | 13.97±0.02$^{f}$ |

All values indicated by different letters are significantly (P<0.05) different between the groups when compared to the control, G1 = Control; G2 = 25mg/l BVE; G3 = 50mg/l BVE; G4 = 100mg/l BVE; G5 = 200mg/l BVE; G6 = 10% $LC_{50}$ AOE; G7 = G2+G6; G8 = G3+G6; G10=G4+G6; G10=G5+G6. Keys: AOE= *Adenium obesum* aqueous extract; BVAE= *Borreria verticillata* aqueous extract.

In this study, there was significant increase in total and differential white blood cells count in G6, possibly in response to tissue damage caused by aqueous extract *Adenium obesum* stem bark. This similar observation has been reported in fish exposed to plant extracts (Olusegun and Adedayo, 2014; Agwu *et al.,* 2018).

There was also a decrease in the values of MCV, MCH, and MCHC, in the acute exposure to AOE, this is similar to the reports of Fafioye (2012) and Baki *et al.* (2015), who reported a significant decrease in all the erythrocytic indices in acute exposure of white tilapia (*Oreochromis niloticus*) to almond (*Terminalia catappa),* pawpaw (*Carica papaya),* Neem (*Azadirachta indica),* tobacco (*Nicotiana tobacum)* and cassava (*Manihot esculenta*) extract. The reduction in levels of these indices may indicate a microcytic hypochromic anemia, the non-responsive nature of the anemia may be due to compromised hematopoietic tissues in the fish such as kidney and spleen.

The observed decrease in the blood indices in G6 (10% $LC_{50}$ AOE) may also be as a result of osmoregulatory imbalances and inhibition in pathways as in the biosynthesis of Hb or inhibiting the utilization of delta-amino levulinic acid (Haux *et al.,* 1985; Nussey *et al.,* 1995; Ajadi *et al*., 2024). The values of erythrocytic indices among the fish treated with AOE were found to decrease with increasing toxicant concentration. Similar decrease has been observed by Okomoda *et al*. (2010).

The values of erythrocytic indices improved with concurrent treatment using *Borreria verticillata* in 7, 8, 9and 10. This can be attributed to a possible ameliorative effect of *Borreria verticillata* in preventing damage to the cell walls of RBCs through its anti-inflammatory action, in a concentration dependent manner (Abdullahi *et al.,* 2014).

WBC is important in regulating the immune system and they usually increase in number as a protective response to oxidative stress. The increase in WBC is suggestive of an adaptive immune response system in the face of stress due to AOE. The increase in WBC in this study is in contrast with the decline reported in WBC reported in *Cyprinus carpio* acutely exposed to atrazine (Blahova *et al.*, 2014) and in *Oncorhynchus mykiss* and *Cyprinus carpio* exposed to metribuzin (Velisek *et al*., 2008, 2009).

The pan leukocytosis induced in fish exposed to *Adenium obesum* extract resulting in the high white blood cell counts recorded in this study could be due to an attempt by the fishes to fight against toxic constituents, with possible production of antibodies to improve the health status of the fish. Similar findings were reported by Ates *et al*. (2008), which reported that the increase in white blood cells count during acute and sub-lethal treatment may be due to stimulated lymphomyeloid tissue as a defense mechanism of the fish to tolerate the toxicity. The increase in lymphocytes count indicates the stimulatory effects of the toxicant on the immune system. Enhancement in the total white blood cell count following exposure to *Adenium obesum* could be possible due to leucocytosis which is an outcome of proliferation of hemopoietic cells, leading to progressive increase in the white blood cell counts in the peripheral blood (Tariq, 2007; Sharma *et al*., 2007). Changes in TWBC and differential count have been reported to play important roles in the assessment of the state of health of fishes, while leucopenia and leukocytosis in *Clarias gariepinus* occur during exposure to pathogens, heavy metals and chemotherapeutics (Omoregie and Oyebanji, 2002).

Various fish species exposed to mercury have shown significant increase in total and differential leucocytes counts ((Ribeiro *et al.,* 2006; Maheswaran *et al.,* 2008; Ibrahim, 2011), similar to the present observation. It can be explained that the stimulation of the immune system causes an elevation in lymphocytes by damages to lymphoid tissues (Shah and Altindag, 2005; Ibrahim, 2011). Kumar *et al.* (2004) had found that exposure to mercuric chloride induced changes in the differential white blood cell counts and caused lymphocytosis, heterophillia, monocytosis, eosinophilia and thrombocytopenia in *Anabas testudineus*, which is similar to the changes reported in this study.

## Conclusion

The fish exposed to *Adenium obesum* stem bark and treated with different concentrations of *Borreria verticillata* aqueous extract, had improved erythrogram values and the 200mg/l BVAE concentration gave the most promising response, where the erythrogram closely mirrored the control group, thus, *Borreria verticillata* extract treatment improved the hematological responses in *Clarias gariepinus* juveniles exposed to the toxicity of *Adenium obesum* stem bark aqueous extract, as such *Borreria verticillata* extract will be a good candidate to ameliorate hemotoxicity due to toxicants in aquatic ecosystems that affects fish such as *Clarias gariepinus*.

## Acknowledgement

The authors are thankful and acknowledge the contribution of Professor Alpha Raj Mekapogu of the Department of Veterinary Pharmacology and Toxicology, College of Veterinary Science, S.V. Veterinary University, Proddatur, India, who provided the guide and mentoring for the toxicological decisions of this study.

## Conflict of Interest

The authors have no conflict of interest to declare.

## Authors Contribution

Conceptualization: MBO, SJS; Literature search: MBO, UAA; Software and Resources: AAA, MBO; Writing of original draft: UAA, AAA; Review of Original draft: SJS, AAA; Data analysis: UAA; Supervision: SJS. All authors reviewed and approved the final draft before submission.

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