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The Haematological Response of *Clariasgariepinus* Juveniles Exposed to Aqueous Bark Extract of *Piptadeniastrumafricanum*

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Abstract: *Fish supply had come majorly from capture. The natural stock continues to go down especially as there is ever increasing demand due to the rising human population. One attempt to reduce the gap between demand and supply is to remove stressors from the natural stock. Piptadenastrium africanum has been frequently used as apiscicide. Investigation was carried out to find how this piscicide influences haematological parameters in juvenile Clariasgariepinus. Different concentration (0.0mg/l, 3.0mg/l, 6.0mg/l, 9.0mg/l, 12.0mg/l, 15.0mg/l) Piptadeniatrum africanum bark extract were dissolved in 40 litres of water in plastic aquaria. Ten fish from a population of Clariasgariepinus of11.5 -14.6 cm total length (mean = 12.45 cm) and 65.6-112.2 g weight (mean = 86.31 g) were randomly selected and cultured in one aquarium with three replicates. Biological and behavioral parameters of the fish including mortality, air gulping, haemorphage, rate of operculation, erratic swimming, discoloration and loss of reflex were monitored in connection with the physicochemical parameters of water. Blood parameters that were investigated include white blood cells count, red blood cells count, platelets count, mean cell haemoglobin, mean cell haemoglobin concentration. Statistical analysis from observation of these parameters means revealed that white blood cell count, mean cell haemoglobin, mean cell haemoglobin concentration were not significantly different. Red blood cells count, haemoglobin, mean cell volume and mean platelets volume showed significant change between the treated and the control groups. For biological and behavioral parameters, there were discoloration of fish skin, air gulping, erratic swimming haemorphage and swimming upside down. Among the physiochemical parameters, measured, dissolved oxygen concentration was significantly lower than in the control groups, temperature and pH were insignificantly changed. The LC50 96 hours of Clariasgariepinus was 6.0mg/L of Piptademastrum africanum. Since blood parameters did not change in a negative direction, death of Clariasgariepinus due to exposure to Piptademanstrumafricanum was attributed to contact action rather than physiological or haematological.*

Keywords: *Piptadeniastrumafricanum, Clariasgariepinus juvenile, haematological response, physicochemical parameters, behavioural and biological parameters*.

1. INTRODUCTION

In Nigeria, fish consumption is lower than world standard or level of consumption. The gap between demand and supply continue to remain wide and effort to narrow this gap is negated by several factors (Fishsite.com, 2014 and Ozigbo *et al*., 2014). Among these factors is the managerial aspect, which includes processes involved in fish capture (Kritzon, 2003).It is therefore difficult for supply to meet demand due to these unrealistic practices. Output from capture fisheries is often lowered by improper gears. Local fishers are aware of potency in stupefying and subsequent killing of fish. In villages, ignorant villagers weaken fish by chemical and biological poisons obtained from stores (gamalin 20) or locally made (e.g small leave and parkia species saw dusts as well as stem and leave concoctions of these plants).(Kritzon, 2003).These gears that are not selective (e.g explosives, poisons and small mesh net) lead to ecosystem over fishing. Some species such as *Clariasgariepinus*among others are those that can help narrow the gap between demand and supply, since *Clariasgariepinus* has been known to be the most popular cultured fish because of its good quality for Aquaculture (Offem *et al*., 2010). It has also been observed that fish always get killed in natural environment where timber operators fall *Piptadeniastrumafricanum* into the water in the process of timber production. How the products of this plant kill fish is not yet understood. Understanding the mechanism the plant kill fish could help prevent further occurrences. By the period such wasteful gears as poisons and explosives are jettisoned in our villages, our streams will be richer with edible ichthyofauna. For reason that, blood is an important tissue of organisms/fish due to the roles it plays in sustaining healthy life

(Adeyemo, 2005; Ajani and Awogbade, 2012; Ayotunde*et al*., 2015; Kori-Siakpere and Ubogu, 2008 and Adakole, 2008), there is need to investigate how this plant affects the haematological parameters.

Much work has been done on *Clariasgariepinus*. This ichthyofauna can be likened to the laboratory rat (*Rathusrathus*) in the mammalian group. Reasons why scientists prefer to work with this fish is the ease with which to handle it. It hardly dies as a result of poor handling and transportation. Apart from this, it has high economic and commercial values. In a work carried out by Nasar*et al* in FAO (2002), *Clariasgariepinus* has the highest commercial values compared to all fishes. They define commercial yield as 'percentual proportion of empty body to total weight of fish'. It was reported that commercial value yield is higher in this fish than in carp species. These workers advised that the highest yield is obtained when the fish is harvested at average weight of 350 g. the high commercial value may have originated from the fish's acceptability in market due to its good eating quality. This has equally made it the third most farmed fish species in Nigeria (Offem*et al*., 2010)

Clarias have no standardized blood pictures in literature as it is for other species (Santhakumer *et al*., 1999; Oluah et al., 2004; Ibiwoye et al., 2006; Palic et al., 2013) in spite of their high advantage as a culture species. Haematological techniques are gaining importance for toxicological research, environmental monitoring and assessment of fish health conditions, (Shah and Altindaga, 2004). Blood parameters are considered patho-physiological indicators of the whole body and therefore are important in diagnosing the structure and functional status of fish exposed to toxicants (Adhikari 2004; Maheswaran et al. 2008). Haematological analysis will enhance fish cultivation by facilitating early detection of situations of stress and or diseases that could affect production performance (Rehulka et al. 2004; Tavares-Dias and Bacellos, 2005). (Kori-Siakpere 1991), experimented on chronic sub-lethal effects of copper in a fresh water teleost, *Clariasisheriensis*; and observed haematological changes resulting from a 90 day exposure to the various sub-lethal concentrations of copper including decrease in haematocrit and haemoglobin values couple with a reduction in erythrocyte counts. (Annune 1998) recorded haematological changes in *Clariasgariepinus* following exposure to sub-lethal concentrations of copper and lead.

Piptadeniastrumafricanum is a commercially valued tree in international trade in Africa. It occurs naturally in almost all the tropical countries of Africa. It is highly valued in African traditional medicine. Its bark is used as a poison in arrow, as ordeal poison, fish poison, as well as poison for mice (Bolza and Keating*.*, 1972). A lot of work has been done on the commercial value of the tree as timber product (Prota base data), in African traditional medicine (Ateulack, *et al.*, 2015) and as fishing gear (Prota base). There is paucity of information on fish blood changes when exposed to products from the tree. This investigation will provide information on blood parameter changes in *Claria. gariepinus* when exposed to bark extract of *Piptadeniastrumatricanum* Therefore this research aims at providing information on the haematological response of *Clariasgariepinus* juvenile exposed to *Piptadeniastrumafricanum* extract, which will form a baseline information on the structure and management of the fisheries in Cross River State and the world as a whole.

2. MATERIALS AND METHODS

2.1. Source of Specimens and Acclimation

Two hundred live and apparently healthy juvenile catfish *Clariagariepinus* measuring 11.5 -14.6 cm total length (mean = 12.45 cm) and 65.6 -112.2 g weight (mean = 86.31 g) were identified by using taxonomic key of (Reed *et al.,* 1967). The specimens used in this experiment were collected from University of Calabar (UNICAL) fish farm. This specimen were kept in a transparent plastic tank and transport to Fisheries laboratory of Cross River University of Technology (CRUTECH) Obubra campus, where they were acclimated for 2 weeks inside a transparent, rectangular glass tank (75×45×45cm) of 121.5L capacity. The tank was filled with 50L unchlorinated stream water. The water was renewed every 2 days and the fish were fed to apparent satiation twice daily with a commercial pelleted fish diet containing 35% crude protein during the acclimation period. Feeding was discontinued 48 hrs before the commencement of the experiment, so as to minimize the production of waste in the test container.

2.2. Physico-Chemical Qualities of Water

Water quality monitoring was carried out before, during and after the experiment using electronic digital meter (Jenway pH Meter model No 3505 made by Bibby Scientific Ltd in UK).

Dissolved oxygen was measured using a digital dissolved oxygen meter (Jenway 970 DO_2Meter produced in People's Republic of Chana) once in a day and this was done at 8.00am while Temperature was measured using a mercury in glass thermometer which was placed in the medium inside the test container until the reading was taken. The reading was taken at 10.00am each day of the experiment.

2.3. Preparation of Piptadeniastrumafricanumaqueous Extract from Bark

the bark of *Piptadeniastrumafricanum* was obtained from Akparabong farm in Ikom Local Government Area of Cross River State, where the tree is commonly found. The bark was peeled using knife/cutlass. The bark was sundried to less than 10 % moisture content before it was pounded into powder. The powder was filtered and stored in polyethylene bag ready for use.

2.4. Behavioural and Biological Observations

Apart from observations and recording fish mortality, the fish behavior such as erratic swimming, air gulping, and loss of reflex, discoloration and molting was also monitored. Death or mortality was defined as a point when fish were no more able to respond to external stimulus such as touch. LC_{50}, which is the concentration of *Piptadeniastrum.Africanum* aqueous extract estimated to be lethal to 50% of the test organism after exposure time of 96hr was determine graphically using probit transformation (Herwig, 1979; USEPA, 2000).

2.5. Toxicity Test

A range finding test was carried out using concentrations from very low to high. The intention of this test was to determine the actual concentration that will be used in the definitive test this test was made of one control and five treated in triplicates. The concentrations were in the strength of 0.0 mg/L, 1.0 mg/L, 10.0 mg/L, 100.0 mg/L, 1000.0 mg/L and 10000.0 mg/L respectively. The behavioural and biological parameters of the test fishes were observed every 15 min for the first hour, once every hour for the next 3 hours and every 4 hours four for the rest 24 hours period. The toxicant concentration was prepared using serial dilution. This is a practice where a stock solution was prepared by dissolving a known grammage of the powder in 4 L of water. Proportionate portions were taken from here to form the required concentration in each case. The definitive was a repetition of the range finding test. The definitive test was selected within the lowest concentration that will kill all the fish within 24 hours. The definitive test experiment was extended for 96 hours. The concentrations were taken as 0.0 mg/L, 1.5 mg/L, 3.0 mg/L, 4.5 mg/L, 6.0 mg/L, 7.5 mg/L and 9.0 mg/L respectively (APHA, 1981). The fish were observed at 30^{th} minute, 1^{st} one hour, 4^{th} hour, 8^{th} hour, 12^{th} hour, 24^{th} hour, 36^{th} hour, 48^{th} hour, 72^{nd} hour and 96^{th} hour respectively.

2.6. Haematological Analysis

About 3 ml of blood was sampled from each triplicate. Blood was removed from a fish by inserting a syringe into the vertebral caudal blood vessel. The blood was then put into 5 ml heparinized blood bottles treated with Ethyl DiamineTetracetic acid (EDTA). The blood samples were transported to University. The Central Laboratory, University of Calabar where they were analysed using a computerized blood analyser (Automated haematology analyser KX-21MTM). The blood parameters analysed were Packed cell volume (PVC), haemoglobin (Hb). Red blood cells count (RBC), white blood cells count (WBC), mean cell volume (MCV), erythrocyte sedimentation rate (ESR), mean cell haemoglobin concentration (MCHC), mean cell haemoglobin (MCH).

2.7. Statistical Analysis

TheLC_{50} was analysed using computerised, probit analysis (Litchfield and Wilcoxon, 1949). Haematological parameters well as physico-chemical properties of water were analysed using one way ANOVA in a computerised statistical package (statistical package for social sciences, SPSS, version 17.0 for windows vista.

3. RESULTS

These were as presented in the following table and figures. Expressed are physicochemical properties of cultured water, biological and behavioural characteristics of fish exposed to extract, LC 50 at different times, red blood cells parameters, platelet size and white blood cells count.

Table1. *The physicochemical properties of water exposed to different concentrations of Pitadenastriumafricanum during range finding and definitive tests.*

Range finding test				Definitive test			
Dose (mg/l)	Dissolved Oxygen (mg/l)	pH	Temp. (^{0}C)	Dose	Dissolved Oxygen (mg/l)	pH	Temp. (^{0}C)
0.0	7.06^{a} ±0.20	7.14±0.22	25	1.5	6.89^{a}±0.56	7.32±0.27	28.67
5.0	6.83^{ab}±0.26	7.01 ±0.46	25	3.0	6.29^{ab}±0.45	7.11±0.25	28.33
10.0	6.76^{ab}±22	6.96±0.32	25	4.5	5.39^{b}±0.49	6.92±0.28	28.00
15.0	6.30^{b}±0.31	7.02±0.19	25	6.0	5.35^{b}±0.44	6.86±0.19	28.33
20.0	6.29^{b}±0.09	6.97±0.28	25	7.5	5.13^{bc}±0.59	7.01±0.19	28.67
25.0	6.25^{b}±0.15	6.93±0.27	25	9.0	4.67^{c}±0.60	6.79±0.16	28.57

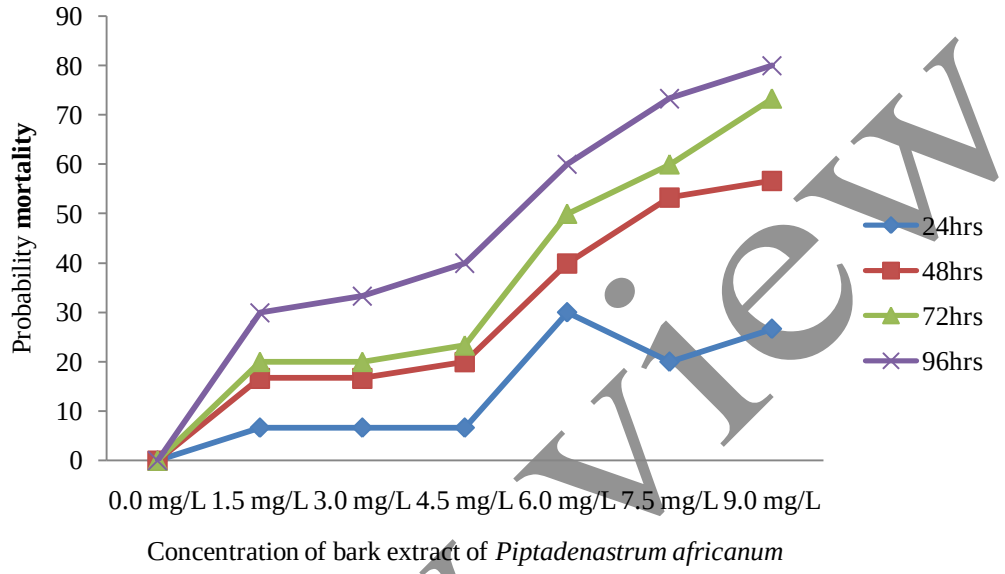

Figure 1. *LC_{50} 30 hrs, 36 hrs, 48 hrs, 72 hrs and 96 hrsof Clariasgariepinus juveniles exposed to Piptadeniastrumafricanum in a definitive test experiment. The LC50 96 hours was 6.0 mg/L; 72 hours was 7.5 mg/L;48 hours was 9.0 mg/L*

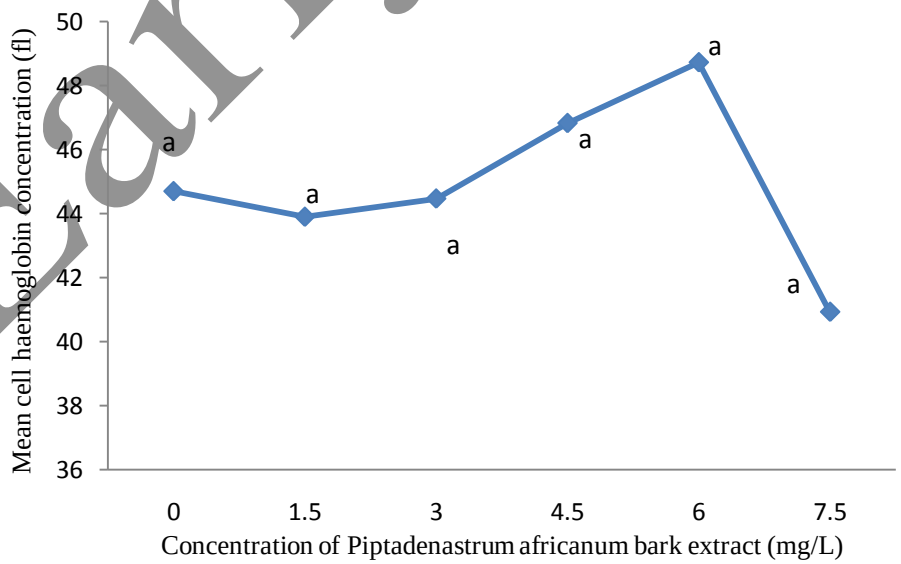

Figure 2. *showing the effects of concentration of Piptadenastrum africanum on the mean cell haemoglobin concentration in Clariasgariepinus juvenile. There were no differences in mean cell haemoglobin concentration (p = 0.05) as observed as the mean value carry same letter.*

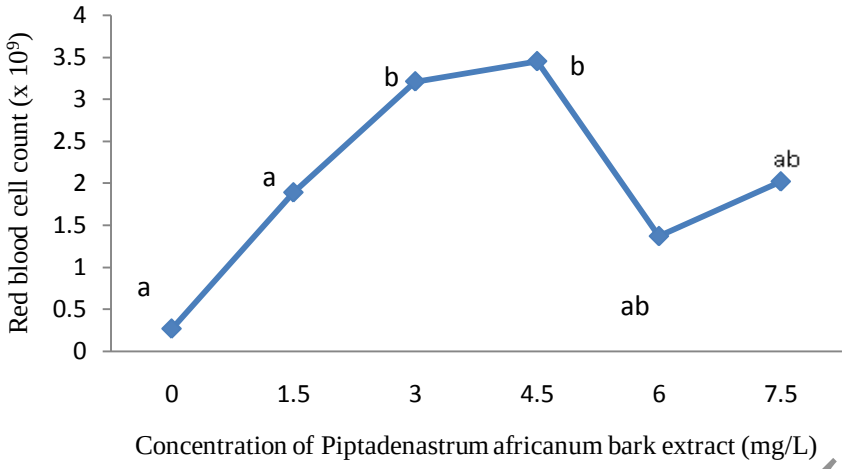

Figure 3. *shows the influence of Piptadenastrum africanum bark extract on red blood cell count in juveniles of Clariasgariepinus. There were differences in red blood cell count between treatments (p = 0.05). Means with same letters are statistically the same while those with different letters are statistically different.*

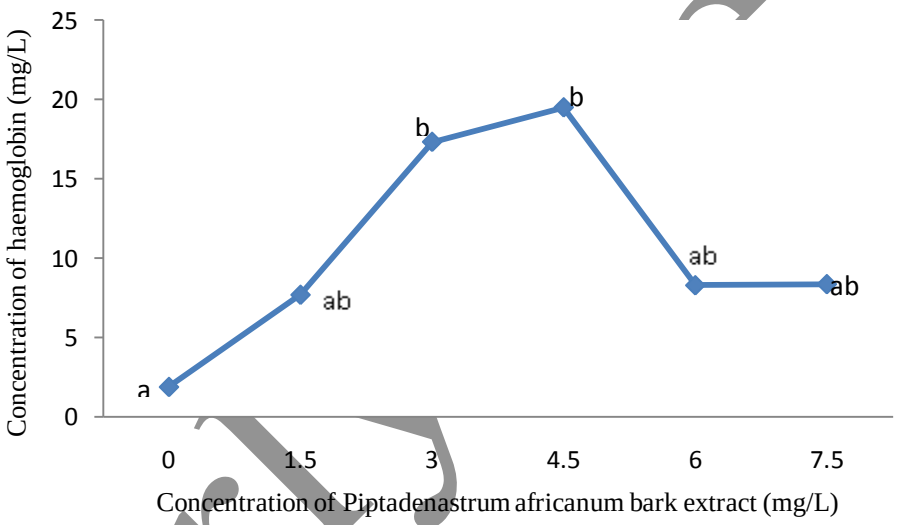

Figure 4. *is aa graph showing haemogobin concentration in juvenile Clariasgariepinusas was affected by Piptadenastrumafricanum bark extract. Treated groups showed more haemoglobin concentration than control. Means with the same letters are statistically the same (p = 0.05), while those means with different letters are statistically different*

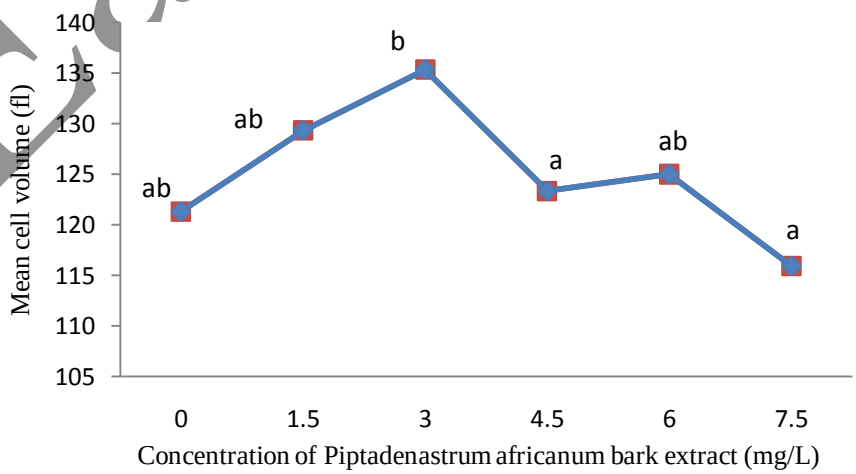

Figure 5. *expresses the mean cell volume in femtoliters, influenced by P. africanum bark extract. Observations show a reduction in cell volume with increasing concentration of Piptadenasstrumbark extract. Similar letters signifies similar means while different letters signifies differences in means.*

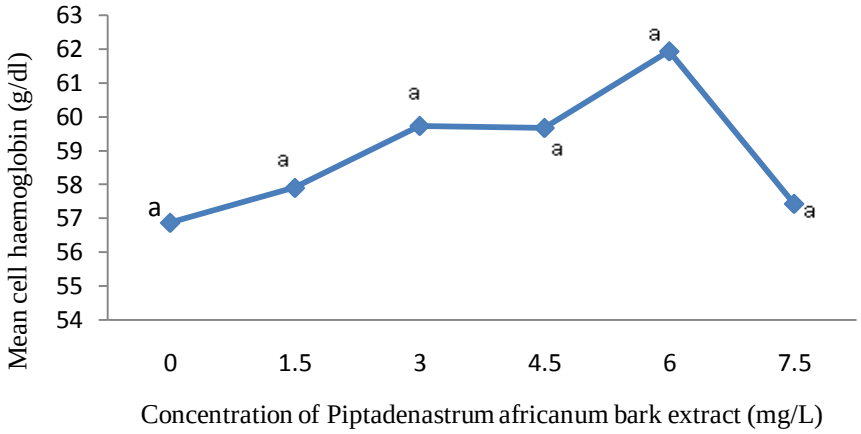

Figure 6. *is the relationship between the concentration of Piptadenastrumafricanum bark extract and mean cell haemoglobin in juvenile Clariasgariepinus. There was no statistical difference between the treated group and the control (p = 0.05).*

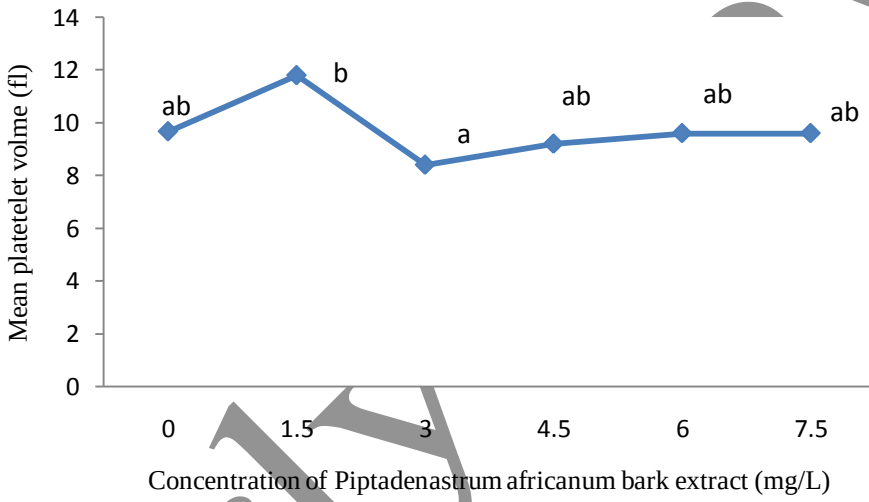

Figure 7. *shows the mean platelete volume, in femtoliters, of Clariasgariepinus juveniles exposed to Piptadenastrumafricanum bark extract, in mg/L. there were difference among means (p = 0.05). Same letters represent statistically similar mean while different letter represent dissimilar means.*

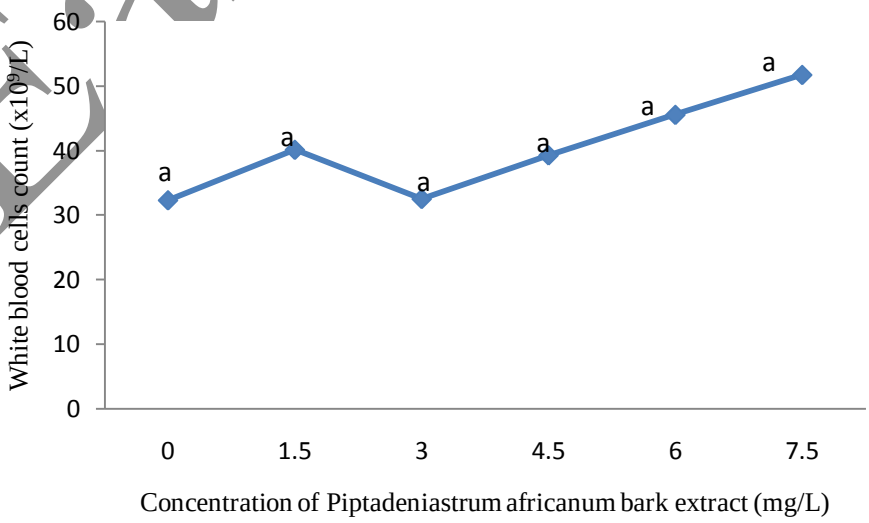

Figure 8. *shows the mean mean total white blood cells count of Clariasgariepinus juveniles exposed to Piptadenastrum africanum bark extract (x10^6/L). There were no differences among means (p = 0.05). Same letters represent statistically similar mean*

Table2. *Showing biological and behavioural responses in Clariasgariepinus exposed to Piptadeniastrum africanus aqueous bark extract during a definitive experiment.*

	6 HOURS						12 HOURS						24 HOURS						36 HOURS						48 HOURS						72 HOURS						96 HOURS					
	0.0 mg/L	3.0 mg/l	30.0 mg/L	300.0 mg/L	3000.0 mg/L	30000.0 mg/L	0.0 mg/L	3.0 mg/l	30.0 mg/L	300.0 mg/L	3000.0 mg/L	30000.0 mg/L	0.0 mg/L	3.0 mg/l	30.0 mg/L	300.0 mg/L	3000.0 mg/L	30000.0 mg/L	0.0 mg/L	3.0 mg/l	30.0 mg/L	300.0 mg/L	3000.0 mg/L	30000.0 mg/L	0.0 mg/L	3.0 mg/l	30.0 mg/L	300.0 mg/L	3000.0 mg/L	30000.0 mg/L	0.0 mg/L	3.0 mg/l	30.0 mg/L	300.0 mg/L	3000.0 mg/L	30000.0 mg/L	0.0 mg/L	3.0 mg/l	30.0 mg/L	300.0 mg/L	3000.0 mg/L	30000.0 mg/L
Loss of reflex	N	N	N	N	Y	Y	N	N	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y
Moulting	N	N	N	Y	Y	Y	N	N	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y
Discoloration	N	N	N	N	N	N	N	N	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y
Air gulping	N	N	N	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y
Erratic swimming	N	N	N	Y	Y	Y	N	N	N	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y
Haemorrhage	N	N	N	N	N	N	N	N	N	Y	Y	Y	N	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y	Y	Y
Swimming upside down	N	N	N	N	N	Y	N	N	Y	Y	Y	Y	N	N	N	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y

Key: N = no response; Y = yes response

4. DISCUSSION

Plant based poisons (phytochemicals) has long over the years been used in catching fish as a gear. These phytotoxinsstupefy fish for the hunters to pick them up with ease. Gupta and Gupta (2006) explained that a chemical, Rotenone is capable of killing fish as a contact poison. It therefore destroys the fish's gill depriving it of oxygen. This lead to fish's death.Velmurugan *et al*. (2009) in their work said that the gills are the primary route for entry of poisons and are critical for respiratory, osmo regulatory and excretory functions. The observation in this work revealed that the toxin equally reduced dissolved oxygen concentration in the medium. Lethality was likely caused by insufficient oxygen in the tissue to carry on normal metabolism (Kepenyes and Varadi, 2016). In a large volume of running water, fish lethality may not necessary be due to low dissolved oxygen concentration but due only to destruction of oxygen absorbing organs, the gills (Kepenyes and Varadi, 2016). This is supported in the fact that the dissolved oxygen concentration did not fall below the lethal concentration (Marie et al., 2015).

Environmental stressors are usually examined in blood pictures of organisms including fish (Adeyemo, 2003 and 2005; Erhunmwunse, and Ainerua, 2013). These stressors do not come in contact with blood directly. Their points of reception are organs which are exposed such as the skin, gills as well as the endothelial tissues like that of the gut. Disturbances in these receptors link the blood, which responds as must have observed in this study. The mean cell haemoglobin and mean cell haemoglobin concentration did not change significantly with the concentration of *Piptadeniastrum africanum* bark extract. However, graphical analysis showed that the insignificant pattern of change resembled that of red blood cells count and haemoglobin concentration.

Red blood cells became more populated in the treated groups. However, at higher concentration of *Piptadeniastrum africanum* extract, the cells population became reduced. Though *Piptadeniastrum africanum* is lethal to fish, it still showed its haemopoeitic ability in this fish (*Clariasgariepinus*).little wonder it has been used in African traditional medicine (Roger et al., 2013). According to Roger et al. (2013), *Piptadeniastrum africanum* extract contains alkaloids, flavonoids, polyphenols, triterpenes, steroids, saponins and tannins, which are important phytomedicines. Haemoglobin concentration followed the trend of red blood cells count, since haemoglobin is a respiratory pigment carried by the red blood cells. The reduction in the mean cell volume observed with increasing concentration of *Piptadeniastrum africanus* agrees with Baker *et al.* (2001) explanation that increased haemopoeisis do lead to higher population of younger cells with smaller volume or size.

White blood cells were not significantly different among the treatments. The number observed in this work falls within the estimation of Erhunmwunse and Ainerua, (2013) of 30.058 to 42.148 x 10^{12} white blood cells per liter of juvenile *Clariasgariepinus* blood. The white blood cells were therefore at their normal range. This means that *Piptadeniastrum africanum* did not exert enough influence on them.. In another work by Ada *et al.* (2016) this plant did not cause pathological alterations in the tissues except the liver that is a detoxifying organ.

Piptadeniastrum africanum actually killed fish. The death of fish, *Clariasgariepinus* exposed to this toxin must have come from other causes other than damages done to its haematocytes. The poison is a contact poison rather than physiological due to the fact that haematological parameters in the exposed specimens were not significantly different from the control. Since it is highly toxic, organs in contact with it get destroyed and the destroyed organs become in capable of transporting it to the others by the blood which is a transporting tissue.

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