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Chronic Toxicity of True Indigo (*Indigofera tinctoria*) Leaf Powder on Antioxidant Status and Haematological Profile of Juveniles African Cat Fish (*Clarias gariepinus*)

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Abstract

The study was conducted to test the toxicity of *Indigofera tinctoria* on the antioxidative status and heamatology of *Clarias gariepinus*. Matured leaves of *I. tinctoria* were collected, dried, and pulverized. Graded levels of the *I. tinctoria* leaf powder were weighed and dissolved in 250 ml of water. Data obtained was subjected to one-way to analyse the significant differences between groups and significance level of $P<0.05$ was used for all tests.

Hematological indices indicated a significant ($P<0.05$) decreasing trend in the red blood cell, haemoglobin and the packed cell volume with increasing concentrations of *I. tinctoria* while the white blood cell counts increased with increasing concentrations of the toxicant. Stress levels inflicted by the test plant were assessed by estimating the effects on the oxidative stress biomarkers in the kidney, liver, muscle and gill of *C. gariepinus*. After the 21 days exposure, activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and catalase (CAT) were investigated and all increased when compared to control. The findings revealed that the disposal of leaves, and effluent from *I. tinctoria* could threaten the life and existence of the aquatic organism.

Introduction

Fish is consumed as food for people around the world, either caught from the wild or cultured (Adesina, 2008). Food fish from aquaculture systems are generally acceptable, and farming procedures must be acceptable to fulfill general, cultural, gender, and social norms, particularly when the result is meant for household consumption (Faturoti, 2000; Adesina, 2008). Freshwater fishes are frequently susceptible to pollution from industrial effluents, surface runoffs which introduces foreign bodies which in turn becomes toxicant that causes physiological problem for the organism (Oladele *et al*., 2005). *Clarias gariepinus* belonging to the Claridae family (Clarias spp.) are highly-valued food fishes in Africa and constitute prominent commercial aquaculture species widely cultivated in Africa, mainly under semi-intensive systems (Fagbenro, 1999). *Clarias gariepinus* are economically important species in Europian countries, Tropical and subtropical nation around the world including Africa and was introduced for intensive culture in some European and Asian countries where feeding constitutes a significant portion of the operating cost (Hecht, 1996).

The plant *Indigofera tinctoria* is commonly used dye raw material in the textile industry for batic and other tye and dye productions popularly called “Adire” in Southwestern Nigeria. It gets into aquatic

environment through direct disposal by artisans and entrepreneurs and industrial effluent discharge (the dye bath) due to proximity of production plant/factories and runoffs into water bodies. Toxicity tests determine the effects of toxic compounds and provide direct proof of organisms' biological responses to pollutants (Eriegha *et al*., 2017). Biochemical characteristics in the cytoplasm and extracellular fluid, such as blood, change, and the cell exposed to toxic substance becomes dysfunctional (Saravanan *et al*., 2011). Haematological features are an important tool that may be used to monitor physiological and pathological changes in fishes as an effective and sensitive measure. Blood parameters are considered patho-physiological indicators of the whole body and are in diagnosing the structural and functional status of fish exposed to various toxicants (Adhikari *et al.,* 2004, Maheswaran *et al.,* 2008). The aquatic biotope, fish species, age, sexual maturity, and health state all influence changes in haematological parameters (Patriche *et al* 2011; Radu *et al*. 2009). Monitoring changes in blood biochemical markers could be a valuable tool for diagnosing pesticide toxicity in target organs and determining the physiological status of pesticide-exposed fish (Banaee *et al*., 2014).

Materials and Method

Preparation of Plant Extract

The matured leaves of *I. tinctoria* were collected from Igbara Oke, Ekiti State. The plant was identified as *I. tinctoria* at the herbarium of the Crop, Soil and Pest Department, The Federal University of Technology Akure using the Plant Catalogue by Odugbemi (2008). The collected *I. tinctoria* leaves were air dried at room temperature 25°C for 2 weeks. After drying, the leaves were pulverized with a sterile manual grinding machine (Binatoe UK) and then sieved with a 100-micron sieve to obtain a fine powder and dissolved in 250 ml of water drawn from the holding tank of the experimental fish.

Test Fish

Three hundred and seventy healthy juveniles each of *C. gariepinus* with a mean weight of 17.02±0.5g were used for this study. The fish was acclimatized for 14 days in dechlorinated water. During acclimation, the fishes were fed twice daily at 0800 and 1600 hours with a commercial floating feed (Ala Aqua) (40% CP) at 3% of their body weight as maintenance ration. The water was changed every 48 hours to prevent the accumulation of toxic waste metabolite.

Experimental Design

The experiment was completely randomized and eighteen (18) glass tanks (40 litres capacity) were used for the experiment. One hundred and eighty *C. gariepinus were* weighed with a top Metler Balance (Metler Toledo, PB8001 London) and randomly distributed into the tank bioassay tanks. Each experimental treatment was replicated. The exposed solution was renewed every 48 hours. The fish were fed twice daily (8:00h – 16:00 h) at 3% body weight with commercial fish feed containing 45% crude protein. Water quality parameters such as Dissolved oxygen, Temperature, Ammonia, Nitrate, Nitrite, Conductivity, pH were also monitored

Sublethal Concentration

Sublethal concentrations were derived to observe various responses of the test fishes to a long term exposure to *I. tinctoria*. In the present study (0, 70, 100, 130, 170, 200 mgL^{-1}) and were selected as sub-lethal concentrations. The fishes were exposed to this varying concentration for 21 days. A control batch was simultaneously experimented with to compare the toxic effect of *I. tinctoria*.

Blood Profile Examination

Fish specimen were anesthezied with clove oil and blood was collected through cardiac puncture with a 2ml heparinzed syringe and dispensed into ethylene-diaminetetraacetic acid (EDTA) bottle to prevent coagulation of blood. The blood profile analyses were carried out in the laboratory according to the method described by Svobodová et al., 1991. Haemoglobin (HB) was performed with the aid of Haemoglobinometer (model AR-740, Sigma®, England), the micro-haematocrit reader was used to measure the packed cell volume (PCV), white blood cell (WBC) and red blood cell (RBC) were counted using the Neubauerhaemocytometer. The red blood cell indices (Mean corpuscular volume (MCV), Mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH)) were calculated.

Antioxidant Enzymes

Superoxide dismutase activity was determined by its ability to inhibit the auto-oxidation of epinephrine by the increase in absorbance at 480 nm. The activity of SOD in the homogenates was determined according to the method described by Misra and Fridovich (1972). The method of Beutler *et al* (1963), and Rotruck *et al* (1973) was adopted in the estimation of the level of glutathione (GSH) and Glutathione perioxidase (GPx). AChE activity was determined with an ultraviolet spectrophotometer from the absorbance changes at 412 nm for 3.0 min at 25°C as described by a modified colorimetric method by Perry *et al* (2000). Catalase activity was determined according to the method described by Mahmoud (2016). Alanine transaminase (ALT) levels was determined following the methods of Reitman and Frankel (1957)

Data/Statistical Analysis

The results of the experiment were expressed as Means and standard deviation (means ± SD), Levene test were used to test the hypothesis of homogeneity respectively, one-way ANOVA method was used to analyze the significant differences between groups. All statistical analysis was carried out with SPSS software (SPSS 22.0, Chicago, IL, United States) and significance level of P<0.05 was used for all tests.

Results

Water Quality Parameters of *C. gariepinus* During 21 Days of Exposure to *I. tinctoria* Leaf Powder

The water quality parameters of *Clarias gariepinus* juveniles to *Indigofera tinctoria* leaf powder are shown in Table 1. The result revealed that there were no significant difference (P>0.05) in the values obtained for temperature and pH among the various treatment groups including the control. A decreasing trend was observed in the dissolved oxygen with increasing concentration of *Indigofera tinctoria* leaf extract. The control (0.00mg/l) had the highest dissolved oxygen value (6.73 mg^{-1}) and the highest concentration (200mg/l) had the lowest value (4.23 mgl^{-1}). The total dissolved solids (TDS) and Electrical conductivity (EC) showed an increasing trend with the increasing concentration of *I. tinctoria* leaf powder. The highest values (193.33 mg L^{-1} and 0.26 μcm^{-1}) respectively were observed in the highest concentration (200 mg/l) and lowest value (146.67 mg L^{-1} and 0.21 μcm^{-1}) in the control group (0.00mg/l). Significant differences (P<0.05) was observed in the values gotten for Total dissolved solid, electrical conductivity and dissolved oxygen. The ammonia, nitrate, and nitrite showed an increasing trend with increasing concentration of the *Indigofera tinctoria* leaf powder. Significant difference were observed in the values obtained among treatments (P<0.05)

Blood Profile of *C. gariepinus* Juveniles During 21 Days Exposure to *I. tinctoria* Leaf Powder

The Blood profile of *C. gariepinus* juveniles exposed to varying concentrations of *I. tinctoria* is presented in Table 2. The red blood cell, haemoglobin and packed cell volume showed a decreasing trend with increasing concentration of *I. tinctoria*, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) values fluctuates, values showed fluctuating trend while an increasing trend was observed in the white blood cell value observed with increasing concentration and time of exposure. A significant difference was observed in the various treatment at P<0.05 when compared with the control.

Table 1. Water quality parameter during 21 days exposure of *Oreochromis niloticus* juvenile to *Indigofera tinctoria*

	CONCENTRATION					
	Control	70	100	130	170	200
TEM	25.13±0.15 ^{a}	25.10±0.12 ^{a}	25.20±0.10 ^{a}	25.03±0.07 ^{a}	24.93±0.07 ^{a}	24.93±0.03 ^{a}
PH	6.76±0.37 ^{a}	6.46±0.03 ^{a}	6.60±0.18 ^{a}	6.83±0.20 ^{a}	7.06±0.16 ^{a}	7.11±0.13 ^{a}
COND	0.21±0.02 ^{a}	0.22±0.01 ^{a}	0.22±0.01 ^{a}	0.25±0.02 ^{ab}	0.26±0.01 ^{b}	0.26±0.01 ^{b}
TDS	146.67±12.02 ^{a}	153.33±6.67 ^{b}	166.33±3.33 ^{c}	173.33±14.53 ^{d}	186.67±3.33 ^{e}	193.33±3.33 ^{e}
DO	6.73±0.88 ^{d}	6.17±0.22 ^{d}	5.70±0.10 ^{c}	4.87±0.77 ^{b}	4.20±0.15 ^{a}	4.23±0.03 ^{a}
AMMONIA	0.20±0.00 ^{a}	0.20±0.00 ^{a}	0.50±0.00 ^{b}	1.00±0.01 ^{c}	1.00±0.01 ^{c}	2.00±0.00 ^{d}
NITRITE	0.25±0.02 ^{a}	0.50±0.02 ^{b}	0.50±0.02 ^{b}	1.00±0.00 ^{c}	1.00±0.00 ^{c}	2.00±0.00 ^{d}
NITRATE	2.50±0.17 ^{a}	5.00±0.17 ^{b}	5.00±0.17 ^{b}	10.00±0.09 ^{c}	20.00±0.04 ^{d}	20.00±0.04 ^{d}

* Values are expressed as mean ± SE. The mean values with different superscript within the same row are significantly different (P<0.05). Temp-Temperature, DO- Dissolved Oxygen, COND- Conductivity, TDS- Total dissolved solid

Table 2. Haematological parameters of *Clarias gariepinus* juvenile exposed to chronic concentrations of *Indigofera tinctoria* leaf powder

	CONCENTRATION mg/l					
Treatment	Control	70	100	130	170	200
PCV	21.00±0.58 ^{f}	19.00±0.58 ^{e}	18.50±0.87 ^{d}	18.00±0.58 ^{c}	17.50±1.44 ^{b}	17.00±0.58 ^{a}
HB	7.00±0.17 ^{d}	6.30±0.17 ^{c}	6.20±0.29 ^{c}	5.95±0.20 ^{b}	5.50±0.46 ^{a}	5.35±0.20 ^{a}
WBC	6.05±0.43 ^{a}	6.80±0.23 ^{b}	7.75±0.38 ^{c}	7.85±0.65 ^{c}	8.35±0.84 ^{d}	8.58±0.30 ^{e}
RBC	2.35±0.06 ^{e}	2.13±0.07 ^{d}	2.0±0.07 ^{c}	1.90±0.00 ^{c}	1.80±0.16 ^{b}	1.70±0.06 ^{a}
MCV	89.36±0.26 ^{a}	89.20±0.32 ^{a}	92.50±7.06 ^{b}	94.74±3.04 ^{c}	97.22±0.09 ^{d}	102.06±0.29 ^{e}
MCH	29.79±.01 ^{a}	29.58±0.19 ^{a}	31.00±0.36 ^{b}	31.31±1.06 ^{b}	30.56±0.06 ^{ab}	31.47±0.15 ^{b}
MCHC	33.33±0.09 ^{bc}	33.16±0.10 ^{b}	33.51±0.01 ^{c}	33.05±0.06 ^{b}	31.43±0.10 ^{a}	31.47±0.06 ^{a}

* Values are expressed as mean ± SE. The mean values with different superscript within the same row are significantly different (P<0.05). HB-haemoglobin, PCV-packed cell volume, RBC-red blood cell, WBC- white blood cell, MCV-mean corpuscular volume, MCH- mean corpuscular haemoglobin, MCHC- mean cell haemoglobin concentration (see **Appendix 1**)

Biochemical Indices in the Gill, Kidney, Liver and Muscle of *C. gariepinus* Juveniles Exposed to *I. tinctoria* Leaf Powder

The biochemical indices of *C. gariepinus* exposed to varying concentration of *I. tinctoria* is shown in Table 3, 4, 5, 6 respectively. There was a significant difference in Catalase activity (CAT), LDH, Superoxide dismutase (SOD), Glutathione content (GSH), Lactate dehydrogenase (LDH), Alanine transaminase (ALT) and inhibition of Ache among the treatment (P<0.05). The result showed an increasing trend with increasing concentration of *I. tinctoria* with the lowest values observed in the control and the highest values observed in the highest concentration.

Table 3. Biochemical indices in the liver of *Clarias gariepinus* during 21 days exposure to *Indigofera tinctoria* leaf powder

	CONCENTRATIONS (mg/l)					
Treatments	Control	70	100	130	170	200
GPX	244.42±0.53^{d}	243.99±0.53^{d}	239.29±0.52^{c}	238.23±0.52^{c}	236.30±0.51^{b}	229.47±0.50^{a}
GSH	36.73±0.08^{e}	28.62±0.06^{a}	29.26±0.06^{b}	31.39±0.07^{c}	33.53±0.07^{d}	38.87±0.08^{f}
SOD	56.73±0.12^{a}	65.46±0.14^{b}	65.46±0.14^{b}	65.46±0.14^{b}	65.46±0.14^{b}	82.92±0.18^{c}
CAT	0.40±0.00^{a}	0.43±0.00^{b}	0.55±0.00^{c}	1.40±0.00^{d}	2.19±0.00^{e}	4.57±0.00^{f}
ALT	11.24±0.02^{a}	20.28±0.04^{b}	28.31±0.06^{c}	31.12±0.07^{d}	33.53±0.07^{e}	33.53±0.07^{e}
LDH	113.76±0.25^{a}	113.76±0.25^{a}	121.88±0.26^{b}	138.14±0.30^{c}	151.94±0.33^{d}	455.03±1.00^{e}
AChE	44.90±0.10^{a}	71.18±0.15^{b}	75.56±0.16^{c}	79.94±0.17^{d}	82.86±0.18^{e}	90.16±0.20^{f}

* Values in the same row with different superscript are significantly different (P<0.05). CAT- Catalase, LDH- Lactate dehydrogenase, SOD- Superoxide dismutase, ALT-Alanine transaminase, GPX-Gluthatione peroxidase, GSH- Gluthatione, AChE- Acetylcholinesterase (see **Appendix 2**)

Table 4. Biochemical indices in the kidney of *Clarias gariepinus* during 21 days exposure to *Indigofera tinctoria* leaf powder

	CONCENTRATIONS mg L^{-1}					
Treatments	Control	70	100	130	170	200
GPX	246.77±0.54^{d}	245.06±0.53^{c}	242.07±0.53^{b}	240.36±0.52^{a}	239.51±0.52^{a}	238.87±0.52^{a}
GSH	27.55±0.06^{a}	30.00±0.06^{b}	32.04±0.07^{c}	33.74±0.07^{d}	35.03±0.08^{e}	35.66±0.08^{f}
SOD	21.82±0.05^{a}	74.19±0.16^{b}	74.19±0.16^{b}	74.19±0.16^{b}	82.92±0.18^{c}	91.65±0.20^{d}
CAT	0.53±0.00^{a}	0.65±0.00^{b}	0.93±0.00^{c}	1.48±0.00^{d}	1.48±0.00^{d}	1.94±0.00^{e}
ALT	7.43±0.02^{a}	8.43±0.02^{b}	10.64±0.02^{c}	12.04±0.03^{d}	23.09±0.05^{e}	29.11±0.06^{f}
LDH	56.88±0.12^{a}	97.51±0.21^{b}	130.01±0.28^{c}	162.51±0.35^{d}	300.65±0.65^{e}	308.77±0.67^{f}
AChE	30.30±0.07^{a}	59.50±0.13^{b}	46.36±0.10^{c}	77.02±0.17^{d}	78.48±0.17^{e}	79.94±0.17^{f}

* Values in the same row with different superscript are significantly different (P<0.05). CAT- Catalase, LDH- Lactate dehydrogenase, SOD- Superoxide dismutase, ALT-Alanine transaminase, GPX-Gluthatione peroxidase, GSH- Gluthatione, AChE- Acetylcholinesterase (see **Appendix 3**)

Table 5. Biochemical indices in the gills of *Clarias gariepinus* during 21 days exposure to *Indigofera tinctoria* leaf powder

	CONCENTRATIONS mg L^{-1}					
Treatments	Control	70	100	130	170	200
GPX	241.00±0.52^{bc}	242.28±0.53^{c}	241.64±0.53^{bc}	240.58±0.52^{bc}	239.93±0.52^{b}	238.01±0.52^{a}
GSH	32.04±0.07^{a}	32.89±0.07^{b}	35.88±0.08^{c}	35.88±0.08^{c}	36.73±0.08^{d}	37.59±0.08^{e}
SOD	24.44±0.05^{a}	30.55±0.07^{b}	39.28±0.09^{c}	56.74±0.12^{d}	65.46±0.14^{e}	74.19±0.16^{f}
CAT	0.53±0.00^{a}	0.54±0.00^{b}	0.60±0.00^{c}	0.64±0.00^{d}	0.84±0.00^{e}	0.88±0.00^{f}
ALT	13.45±0.03^{a}	16.66±0.04^{c}	16.26±0.04^{b}	17.06±0.04^{d}	17.47±0.04^{e}	19.87±0.04^{f}
LDH	178.76±0.39^{a}	203.14±0.44^{b}	219.39±0.48^{c}	227.52±0.49^{d}	235.64±0.51^{e}	268.144±0.58^{f}
AChE	18.62±0.04^{a}	20.08±0.04^{b}	24.46±0.05^{c}	47.82±0.10^{d}	58.04±0.13^{e}	63.88±0.14^{f}

* Values in the same row with different superscript are significantly different (P<0.05). CAT- Catalase, LDH- Lactate dehydrogenase, SOD- Superoxide dismutase, ALT-Alanine transaminase, GPX-Gluthatione peroxidase, GSH- Gluthatione, AChE- Acetylcholinesterase (see **Appendix 4**)

Table 6. Biochemical indices in the muscle of *Clarias gariepinus* during 21 days exposure to *Indigofera tinctoria* leaf powder

	CONCENTRATIONS mg L^{-1}					
Treatments	Control	70	100	130	170	200
GPX	243.57±0.53^{c}	243.14±0.53^{c}	242.92±0.53^{c}	239.72±0.52^{c}	238.44±0.52^{ab}	237.80±0.52^{a}
GSH	26.06±0.06^{a}	32.25±0.07^{b}	34.17±0.07^{c}	34.38±0.07^{c}	36.52±0.08^{d}	36.52±0.08^{d}
SOD	57.74±0.13^{a}	65.46±0.14^{b}	74.19±0.16^{c}	74.19±0.16^{c}	74.19±0.16^{c}	74.19±0.16^{c}
CAT	0.39±0.00^{a}	0.41±0.00^{b}	0.46±0.00^{c}	1.03±0.00^{d}	1.03±0.00^{d}	4.19±0.01^{e}
ALT	4.02±0.01^{a}	8.63±0.02^{b}	9.23±0.02^{c}	9.84±0.02^{d}	23.29±0.05^{e}	31.32±0.07^{f}
LDH	292.52±0.64^{a}	390.03±0.85^{c}	381.90±0.83^{b}	422.53±0.92^{d}	422.53±0.92^{d}	446.91±1.00^{e}
AChE	24.46±0.05^{a}	31.76±0.07^{b}	52.20±0.11^{c}	60.96±0.13^{d}	68.26±0.15^{e}	73.73±0.16^{f}

*Values in the same row with different superscript are significantly different (P<0.05). CAT- Catalase, LDH- Lactate dehydrogenase, SOD- Superoxide dismutase, ALT-Alanine transaminase, GPX-Gluthatione peroxidase, GSH- Gluthatione, AChE- Acetylcholinesterase (see **Appendix 5**)

Discussion

The results obtained from this study showed a decrease in RBC, Hb, and PCV and an increase in WBC during chronic exposure to *I. tinctoria* leaf powder (P≤0.05). Generally, the PCV value depends on the oxygen-carrying capacity of the blood (Larsson *et al.,* 1985). In the present study the observed decrease in PCV value may be due to the less oxygen content in the blood of fish. A. similar trend was observed by Adeyemo, (2005) who worked on the effect of cassava effluent on the heamatology and histology of *C. gariepinus* and Aderolu *et al.,* (2010) who worked on the effect of acute and sub-lethal concentration of Actellic on weight and heamatology of *C. gariepinus* and Okomoda and Ataguba (2011) who worked on the acute toxicity of *Clarias gariepinus* exposed to Sunsate. Moreover, lower PCV values also indicate shrinkage of cell due to toxicant stress on erythropoietic tissue (Saravanan *et al.,* 2011), A progressive change in fish hematological parameters occurred, due to physiological stress in fish, probably. The *I. tinctoria* leaf powder can affect RBC, causing hemolysis by a disruptive effect on the erythropoietic tissues. The decrease in hemoglobin concentration may be due to either an increase in the rate at which hemoglobin is destroyed or a decrease in the rate of hemoglobin synthesis. The erythrocyte indices (MCV, MCH, and MCHC) obtained in the present study confirms that exposure to *I. tinctoria* leaf powder can stimulate erythropoiesis in *C. gariepinus*. Hedayati and Jahanbakhshi (2013) reported a similar result in Great sturgeon (*Huso huso*) exposed to crude oil for 48 h and 7 days caused a decrease in PCV and haemoglobin due to haemolysis. A similar trend was observed by Simonato *et al.,* 2006; Ramesh *et al*. 2014; Joshi *et al.,* 2002; Adedeji *et al.,* 2010; Kavitha *et al.,* 2010). Similar Blood profiles were detected in RBC, Ht, and Hb of olive flounder (*Paralichthys olivaceus*) exposed to a single PAH, phenanthrene (Jee *et al.,* 2004). Also, a decrease in the RBC level of *Siganus rivulatus* at acute exposure to a high concentration of crude oil (Eisler, 1975). Gaafar *et al.,* (2010) reported that prolonged reduction in haemoglobin content is deleterious to oxygen transport and degeneration of the erythrocytes could be due to pathological condition in fish exposed to toxicants.

Similar observations have also been reported in a study on the exploitation of ethanol extract of *Adenium obesum* stem bark as a potent organic piscicide (Abalaka *et al.,* 2014). In a study conducted by Olusegun and Adedayo (2014) on blood profiles, serum biochemistry and histology of *C. gariepinus*, exposed to sub-lethal concentrations of cold water fresh root bark extract of *Plumbago zeylanica* (Leadwort) for 21 days resulted in remarkable reduction of (RBC, Hb, PCV) leading to an anaemic condition in *C. gariepinus* juvenile. In this study, increase in WBC confirms concentration-related toxicity of the toxicant to the test organism. Gabriel *et al.,* 2007 reported a similar trend on exposure of *C. gariepinus* exposed to refined oil and kerosine under laboratory conditions, also Okogwu *et al.,* 2015 observed an increase in WBC count on exposure of *C. gariepinus* to 2,4-dichlorophenoxyacetic acid (2,4-D). Olusegun and Adedayo (2014) and Adeyemo (2005) reported an increase in total WBC when different fish species and *C. gariepinus* were exposed to *Plumbago zeylanica* extract and cassava effluent respectively. The increase in the values of ALT of test fish reported herein corroborates the findings of Luskova et al. (2002) when *Cyprinus carpio* was exposed to diazinon but contradicts Tiwari and Singh (2004) study where *Channa punctatus* was treated with sublethal levels of alcoholic extracts of *Nerium indicum*. The result obtained from this study showed a significant increase in the LDH contained in the Liver, Kidney, gill and Muscle of *C. gariepinus* infers that the tissues were damaged as a result of the increasing concentration of *I. tinctora* which is in line with the work of Audu *et al.,* 2015 who investigated the effect of varying concentrations of *Cannabis staiva* leaf extract on biochemical changes and alteration level in Gill, liver and serum of *Cyprinus carpio* during the 56 days experiment. Nwani *et al.,* 2016 reported a significant increase in the CAT activity of *C. gariepinus* exposed to *Psychotria* microphylla leaf extract for 15 days and stated that the significant increase observed showed that the leaf extract caused oxidative stress in the exposed fish and this corroborated with the result of this study.

The SOD values obtained in this study corroborates with the work of Ekeh *et al.,* 2018 who reported a significant increase in the SOD activity of *C. gariepinus* exposed sublethal concentration of potassium dichromate and also when chlorpyrifos caused on oxidative stress on the gill, liver and kidney of *Ctenopharyngodon idellus* (Kaur and Jindal, 2017).

Conclusion

This study revealed that *I. tinctoria* when released into the aquatic environment at 170 mgL^{-1} concentration had a negative effects on the health status of Juveniles African cat fish (*Clarias gariepinus*), the resulting blood and biochemical result showed that the fish exposed are stressed and longer exposure or increased concentration will lead to the eventual death. This suggested that environmental risk assessment linked with *I. tinctoria* uses would be required. 170 mgL^{-1} concentrations of the leaf powder induced changes in the haematological and biochemical indices in the liver, kidney, gill and muscle of the fish species. Therefore, disposal of leaves and effluent from *I. tinctoria* plant into water bodies should be done with extreme caution as it could threaten the life and existence of fish

Ethical Statement

The experiment was carried out according to the guidelines for animal welfare and ethics as prescribed by the Federal University of Technology, Akure Nigeria Animal Ethics Committee.

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Author Contribution

Conceptualization: All authors, Data Curation: First and Second author, Formal analysis: First author, Investigation: First author, Methodology: First and Second author, Visualization: Second, Third and Fourth author, Supervision: Second, Third and Fourth author, Writing original draft: First author, Review and Editing: Second, Third and Fourth author.

Conflict of Interest

The authors declared that they have no known competing conflict either financial or non-financial, professional or personal that could have appeared to influence the work reported in this paper.

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Appendix 1. Test of Homogeneity of Variances for Haematology

	Levene Statistic	df1	df2	Sig.
PCV	.704	5	12	.631
HB	.687	5	12	.642
WBC	.830	5	12	.552
RBC	1.486	5	12	.265
MCV	3.485	5	12	.035
MCH	3.384	5	12	.039
MCHC	.939	5	12	.490

Appendix 2. Test of Homogeneity of Variances for Biochemical Indices in the Liver

	Levene Statistic	df1	df2	Sig.
GPX	.002	5	12	1.000
GSH	.061	5	12	.997
SOC	.065	5	12	.996
CAT	2.229	5	12	.119
ALT	.416	5	12	.829
LDH	1.495	5	12	.263
AChE	.173	5	12	.968

Appendix 3. Test of Homogeneity of Variances for Biochemical Indices in the Kidney

	Levene Statistic	df1	df2	Sig.
GPX	.001	5	12	1.000
GSH	.037	5	12	.999
SOC	.448	5	12	.807
CAT	.749	5	12	.602
ALT	1.068	5	12	.425
LDH	1.108	5	12	.406
AChE	.397	5	12	.842

Appendix 4. Test of Homogeneity of Variances for Biochemical Indices in the Gill

	Levene Statistic	df1	df2	Sig.
GPX	.000	5	12	1.000
GSH	.016	5	12	1.000
SOC	.597	5	12	.703
CAT	.201	5	12	.956
ALT	.060	5	12	.997
LDH	.073	5	12	.995
AChE	.885	5	12	.520

Appendix 5. Test of Homogeneity of Variances for Biochemical Indices in the Muscle

	Levene Statistic	df1	df2	Sig.
GPX	.000	5	12	1.000
GSH	.054	5	12	.998
SOC	.039	5	12	.999
CAT	2.576	5	12	.083
ALT	1.480	5	12	.267
LDH	.076	5	12	.995
AChE	.525	5	12	.753