E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 76.37
(GIF) Impact Factor: 0.549
IJFAS 2022; 10(5): 50-56

www.fisheriesjournal.com
Received: 20-05-2022
Accepted: 22-06-2022

Olowolafe Tunde
Department of Fisheries and Aquaculture Technology, The Federal University of Technology Akure, PMB 704, Akure, Nigeria

Popoola Omoniyi M
Department of Fisheries and Aquaculture Technology, The Federal University of Technology Akure, PMB 704, Akure, Nigeria

Fagbenro Oyedapo A
Department of Fisheries and Aquaculture Technology, The Federal University of Technology Akure, PMB 704, Akure, Nigeria

Olawusi-Peters Olaronke O
Department of Fisheries and Aquaculture Technology, The Federal University of Technology Akure, PMB 704, Akure, Nigeria

Corresponding Author:
Olowolafe Tunde
Department of Fisheries and Aquaculture Technology, The Federal University of Technology Akure, PMB 704, Akure, Nigeria

Toxicity of true indigo (*Indigofera tinctoria*) extract on haematology and oxidative stress enzymes of Nile tilapia (*Oreochromis niloticus*) juveniles

Olowolafe Tunde, Popoola Omoniyi M, Fagbenro Oyedapo A and Olawusi-Peters Olaronke O

DOI: https://doi.org/10.22271/fish.2022.v10.i5a.2729

Abstract
This study was conducted to ascertain the toxicity effect of *Indigofera tinctoria* on haematology and oxidative stress biomarker on the liver, kidney, gills, and tissue of Nile tilapia (*Oreochromis niloticus*) juveniles. Matured leaves of *Indigofera tinctoria* were collected, dried, and pulverized. Graded levels of the *Indigofera tinctoria* leaf powder were weighed and dissolved in 250 ml of water. Fish specimens were exposed to sub-acute concentrations of *Indigofera tinctoria* for 21 days. Significant changes and dose-dependent decreases in red blood cells, packed cell volume, hemoglobin values, and progressive increase in white blood cells were observed in *Indigofera tinctoria* exposed fish. There was an elevation in the activities of oxidative, in the liver, kidney, gill and tissue of the fish. The present findings show that disposal of leaves, effluent from *Indigofera tinctoria* should be done with extreme caution as it could threaten the life and existence of the aquatic organism.

Keywords: sub-acute, biomarker, oxidative stress, transaminase, *Indigofera tinctoria*

1. Introduction
Freshwater fishes are frequently susceptible to pollution from industrial effluents, according to Oladele *et al.* [1] Industries find it more cost-effective to release their waste into streams and rivers that run into larger bodies of water and pollute the water. High quantities of several trace metals were found in local freshwater systems, particularly rivers, according to Omoregie *et al.* [2] Toxicants introduced into an aquatic environment cause physiological problems in aquatic creatures [3]. Metals are present in most industrial effluents, some of which are hazardous to living species, including fish [4]. The Nile Tilapia, *Oreochromis niloticus*, is a member of the Cichlidae family. *Oreochromis niloticus*, a squat-shaped tilapia, is described as the finest species for culture among the tilapia family. According to Fagbenro [5], tilapia species are important economically in tropical and sub-tropical nations around the world, particularly in Africa, where mixed-sex tilapia are used in production ponds. For a long time, animal toxicity tests have been used to determine the potential danger posed by toxicants to humans [4]. The use of *Indigofera tinctoria* (local dye) is commonly used by the textile industry to dye fabrics (adire), after which the dye bath (effluent) is discharged especially into the drainage canals leading to streams and rivers, endangering aquatic life. It is known to contain some piscicidal compounds that can be lethal to freshwater fish. Therefore, it is of utmost importance to conduct toxicity tests to determine the extent of harm and adverse effects to fish species and other aquatic life when the extracts of *Indigofera tinctoria* are discharged into the aquatic environment.

2. Materials and Method
2.1 Extraction and preparation of the plant extract (*Indigofera tinctoria*)
The mature leaves of *Indigofera tinctoria* were collected from the top of the plant. The collected *Indigofera tinctoria* leaves were air dried at room temperature (25 °C) for 2 weeks. The dried leaves were pulverized using a sterile hand mill (Binatoe UK) and then sieved using a 100-micron sieve to obtain a fine powder. Graded amounts (1.0 g, 2.0 g, 3.0 g, 4.0 g, and 5.0

g) of *Indigofera tinctoria* was dissolved in 250 ml of water

2.2 Test fish
Three hundred and seventy apparently healthy juvenile *Oreochromis niloticus* fish with an average weight of 16.4±0.29 were used for this study. Fish were acclimated in DE chlorinated water for 14 days and were fed a commercial floating diet (Ala Aqua) (40% CP) at 3% of their body weight twice daily at 0800 and 1600 h as a maintenance ration. The water was changed every 48 hours to prevent accumulation of toxic waste.

2.3 Sub lethal concentration
Sub lethal or safe concentrations were derived to observe different responses of the test fish during prolonged exposure to *Indigofera tinctoria*. In the present study, (0.2, 0.4, 0.6, 0.8, 1.0 g) were selected as sub lethal concentrations for *Oreochromis niloticus*. Fish were exposed to these different concentrations for a period of 21 days. A control batch corresponding to the test group was studied simultaneously to compare the toxic effect of *Indigofera tinctoria*.

2.4 Experimental design
The experiment was completely randomized. Eighteen (18) plastics tanks (40 liters) were used. One hundred and eighty (180) *Oreochromis niloticus* juveniles were weighed and randomly distributed into the control and treatment bioassay tanks separately at a stocking rate of ten fish per tank. Each experimental treatment was replicated. Water quality of the test media was monitored and sampled daily while remnants of the unconsumed feed and the excreta were also siphoned. 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.

2.5 Blood profile Examination
2.5.1 Fish Blood Collection and Analysis
Fish specimens were anaesthetized with clove oil and a 2ml heparinized syringe was used to collect blood through caudal puncture and dispensed into Ethylenediamine tetraacetic acid (EDTA) bottle to prevent coagulation of blood. Blood samples were transferred to the laboratory for haematological analysis. Haemoglobin, red blood cell, white blood cell, packed cell volume were examined. The absolute values made up of mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), and mean corpuscular volume (MCV) were calculated from the results of Erythrocyte/RBC, haemoglobin (Hb), and PCV/ (Ht)

2.6 Oxidative stress enzymes and Lipid Peroxides Determination
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 of Perry *et al.* (2000) [6]. 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 [7]. Catalase activity was determined according to the method described by Mahmoud [8]. The method of Beutler *et al.* [9], and Rotruck *et al.* [10] was adopted in the estimation of the level of glutathione (GSH) and Glutathione perioxidase (GPx).

2.7 Determination of Transaminase Enzymes Responses
Alanine transaminase (ALT) levels was determined following the methods of Reitman and Frankel [11]

2.8 Data/Statistical Analysis
The results of the experiment were expressed as Means and standard deviation (means ± SD), Kolmogorov–Smirnov test was used to test the hypothesis of normality and homogeneity respectively, one-way ANOVA method was used to analyse the significant differences between groups. All statistical analysis was carried out with SPSS software (SPSS 22.0, Chicago, IL, United States) and a significance level of $p < 0.05$ was used for all tests.

2.9 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.

3. Results
3.1. Water quality parameters of *Oreochromis niloticus* exposure to *Indigofera tinctoria* aqueous extract
The result revealed that there was no significant difference ($p>0.05$) in the values obtained for temperature and pH among the various treatment as well as the control. The total dissolved solids (TDS) and Electrical conductivity (EC), Dissolved Oxygen (DO) increased with increasing concentration of *Indigofera tinctoria* aqueous extract. The ammonia, nitrate, and nitrite showed an increasing trend with increasing concentration of the *Indigofera tinctoria* leaf extract. Significant difference was observed in the values obtained among treatments ($p<0.05$) (Table 1)

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

	Concentration mg L^{-1}					
	Control	**13**	**27**	**40**	**53**	**67**
Temp (^oc)	25.27±0.03 ^{a}	25.33±0.09 ^{a}	25.37±0.03 ^{a}	25.37±0.03 ^{a}	25.30±0.00 ^{a}	25.20±0.06 ^{a}
pH	6.87±0.02 ^{a}	6.66±0.03 ^{a}	6.64±0.03 ^{a}	6.60±0.01 ^{a}	6.57±0.02 ^{a}	6.54±0.01 ^{a}
DO (mg l^{-1})	8.30±0.44 ^{d}	8.10±0.20 ^{d}	7.30±0.12 ^{c}	7.50±0.06 ^{c}	6.90±0.00 ^{b}	5.80±0.06 ^{a}
Cond(µcm^{-1})	0.17±0.00 ^{a}	0.20±0.00 ^{b}	0.29±0.00 ^{b}	0.31±0.00 ^{b}	0.39±0.00 ^{c}	0.41±0.00 ^{c}
TDS (mg l^{-1})	116.67±3.33^a	133.67±3.33^b	133.33±3.33^b	136.67±3.33^c	136.33±3.33^c	143.33±3.33^d
Ammonia (mg l^{-1})	0.00±0.00 ^{a}	0.20±0.00 ^{b}	0.50±0.02 ^{c}	0.50±0.00 ^{c}	1.00±0.00 ^{d}	2.00±0.00 ^{e}
Nitrite (mg l^{-1})	0.25±0.02 ^{a}	0.25±0.02 ^{a}	0.50±0.02 ^{b}	1.00±0.01 ^{c}	2.00±0.00 ^{d}	4.00±0.00 ^{e}
Nitrate (mg l^{-1})	2.50±0.17 ^{a}	5.00±0.17 ^{b}	10.00±0.17 ^{c}	10.00±0.09 ^{c}	20.00±0.04 ^{d}	40.00±0.02 ^{e}

*Values in the same row with different superscript are significantly different ($p<0.05$)
Temp- Temperature DO- Dissolved Oxygen COND- Conductivity TDS- Total dissolved solid

3.2. Blood profile of *Oreochromis niloticus* juveniles during 21 days exposure to *Indigofera tinctoria* aqueous extract

The red blood cell, haemoglobin and packed cell volume showed a decreasing trend with increasing concentration of *Indigofera tinctoria*, an increasing trend was observed in the white blood cell value observed with the increasing concentration. Significant difference was observed in the various treatment at $p<0.05$ when compared with the control. The control had the highest HB, PCV, RBC (4.90g/dl, 14.50%, 1.65×10^{12}) respectively and the highest treatment had the lowest values (3.20g/dl, 11.00%, 1.03×10^{12}) respectively. The WBC of the control (0.00 mg/l) had the lowest values 6.15×10^{3}/L and it increased with increasing concentration with the highest concentration (170 mg/l) value to be 9.60×10^{3}/L (Table 2).

Table 2: Hamatological parameters of *Oreochromis niloticus juvenile* exposed to *Indigofera tinctoria* aqueous extract

	Concentration mg/l					
Treatments	**Control**	**13**	**27**	**40**	**53**	**67**
HB	4.90±0.12 ^{d}	4.20±0.29 ^{c}	4.10±0.58 ^{c}	3.75±0.09 ^{b}	3.60±0.29 ^{b}	3.20±0.17 ^{a}
PCV	14.50±0.29 ^{e}	13.50±0.87 ^{e}	13.00±1.73 ^{d}	12.90±0.29 ^{c}	12.20±0.29 ^{b}	11.00±0.58 ^{a}
WBC	6.15±0.14 ^{a}	8.00±0.07 ^{b}	8.05±0.89 ^{b}	8.20±0.35 ^{c}	8.25±0.20 ^{c}	9.60±0.12 ^{d}
RBC	1.65±0.03 ^{d}	1.43±0.10 ^{c}	1.40±0.19 ^{c}	1.30±0.04 ^{b}	1.20±0.06 ^{a}	1.15±0.07 ^{a}
MCV	87.87±0.21 ^{a}	94.40±0.14 ^{ab}	92.86±0.56 ^{a}	99.23±0.64 ^{c}	101.67±1.63 ^{c}	95.65±0.56 ^{b}
MCH	29.69±0.18 ^{b}	29.37±0.07 ^{b}	29.29±0.22 ^{b}	28.84±0.28 ^{a}	30.00±3.50 ^{bc}	36.80±0.38 ^{c}
MCHC	33.79±0.12 ^{c}	31.11±0.02 ^{b}	31.54±0.04 ^{b}	29.07±0.07 ^{a}	29.51±3.20 ^{a}	29.09±0.22 ^{a}

*Values in the same row with different superscript 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

3.3 Oxidative stress enzyme response in the gill of *Oreochromis niloticus* exposed to varying concentrations of *Indigofera tinctoria* aqueous extract

In the treated group the ALT activity was elevated above the control in all the concentrations, fish exposed to the highest concentration 67 mg/l, the elevation was 69.50% (16.46 ± 0.04 UI/L) above the control (5.02 ± 0.01 IU/L). The *Indigofera tinctoria* aqueous extract caused an increase in CAT activity with the lowest value, 1.39±0.00 IU/L recorded at 13 mg/l compared with the control, 0.51±0.00 IU/L. SOD activity was elevated from 30.55±0.07 IU/L in the control (0.00 mg/l) to 74.19±0.16 IU/L at 67 mg/l of *Indigofera tinctoria* aqueous extract. LDH activity was elevated by 4.00%, and 35.14% in fish exposed to 13 mg/l and 67 mg/l of *Indigofera tinctoria* aqueous extract respectively, above the control, 195.01±0.42 IU/L. GPX activity in the gill was elevated in all the exposed concentrations and the highest 246.34±0.54 was observed in 67 mg/l, above the control, 241.86±0.53 IU/L. Significant increase in GSH was observed in all treatment with 8.46% at the highest concentration 67 mg/l above the control. Elevation was also recorded for AChE with 57.7% excitation at 67 mg/l above the control, 25.38±0.06 IU/L (Table 3)

Table 3: Biochemical indices in the gill of *Oreochromis niloticus* during 21 days exposure to *Indigofera tinctoria* aqueous extract

	Concentrations mg L^{-1}					
Treatments	**Control**	**13**	**27**	**40**	**53**	**67**
CAT	0.51±0.00^{a}	1.39±0.00 ^{b}	1.88±0.00 ^{c}	2.11±0.00 ^{d}	2.11±0.00 ^{d}	5.86±0.01^{e}
LDH	195.01±0.42^{a}	203.14±0.44^{b}	260.02±0.57^{c}	284.40±0.62^{d}	300.65±0.65^{e}	373.78±0.81 ^{f}
SOD	30.55±0.07 ^{a}	39.28±0.09 ^{b}	56.74±0.12 ^{c}	56.74±0.12 ^{c}	74.19±0.16 ^{d}	74.19±0.16 ^{d}
ALT	5.02±0.01 ^{a}	5.42±0.01 ^{b}	8.83±0.02 ^{c}	10.24±0.02 ^{d}	16.46±0.04 ^{e}	17.47±0.04 ^{f}
GPX	241.86±0.53^{a}	243.78±0.53^{b}	244.21±0.53^{b}	244.85±0.53^{b}	246.34±0.54^{c}	246.55±0.54 ^{c}
GSH	16.23±0.04 ^{a}	17.09±0.04 ^{b}	17.94±0.04 ^{c}	17.51±0.04 ^{d}	17.73±0.04 ^{e}	18.15±0.04 ^{f}
AChE	25.38±0.06 ^{a}	46.15±0.10 ^{b}	50.19±0.11 ^{c}	53.07±0.12 ^{d}	60.00±0.13 ^{e}	61.15±0.13 ^{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 transaminaseGPX-Gluthatione peroxidase GSH-Gluthatione AChE- Acetylcholinesterase

3.4. Oxidative stress enzyme response in the kidney of *Oreochromis niloticus* exposed to varying concentrations of *Indigofera tinctoria* aqueous extract

In the treated group the ALT activity was elevated above the control, 2.01±0.04 IU/L in all the concentration. *Indigofera tinctoria* aqueous extract caused an increase in CAT activity with the lowest value, 5.86±0.01 IU/L recorded at 13 mg/l and the highest 8.79±0.02 IU/L at 67 mg/l. SOD activity was elevated from 21.82±0.05 IU/L in the control to 65.46±0.14IU/L at 67 mg/l of *Indigofera tinctoria* aqueous extract. LDH activity was excited by 10.72%, and 21.88% in fish exposed to 13 mg/l and 67 mg/l of *Indigofera tinctoria* aqueous extract respectively, above the control, 203.14±0.44 IU/L. GPX activity in the kidney was elevated at in all the exposed concentration and the highest 246.34±0.54 was observed in 67 mg/l, above the control, 241.86±0.53 IU/L. Significant increase in GSH was observed by 5.76% at the highest concentration 67 mg/l above the control. Elevation was also recorded for AChE with 57.38% excitation at 67 mg/l above the control, 28.27±0.06 IU/L (Table 4).

Table 4: Biochemical indices in the kidney of *Oreochromis niloticus* during 21 days exposure to *Indigofera tinctoria* aqueous extract

	Concentration mg L^{-1}					
Treatments	**Control**	**13**	**27**	**40**	**53**	**67**
CAT	5.86±0.01 [a]	5.86±0.01 [a]	6.59±0.01 [b]	6.59±0.01 [b]	8.79±0.02 [c]	32.64±0.08 [d]
LDH	203.14±0.44 [a]	227.52±0.49 [b]	227.52±0.49 [c]	251.89±0.55 [c]	260.02±0.57 [d]	300.65±0.65 [e]
SOD	21.82±0.05 [a]	21.82±0.05 [a]	30.55±0.07 [b]	56.74±0.12 [c]	65.46±0.14 [d]	91.65±0.20 [e]
ALT	2.01±0.04 [a]	2.81±0.01 [b]	3.61±0.01 [c]	4.82±0.01 [d]	5.62±0.01 [e]	7.83±0.02 [f]
GPX	243.78±0.53 [a]	244.85±0.53[ab]	245.06±0.53[ab]	245.34±0.54 [b]	245.70±0.53 [b]	245.70±0.53 [b]
GSH	17.51±0.04 [a]	17.73±0.04 [b]	18.15±0.04 [c]	18.37±0.04 [d]	18.58±0.04 [e]	19.00±0.04 [f]
AChE	28.27±0.06 [a]	59.42±0.13 [b]	61.73±0.13 [c]	65.18±0.14 [d]	66.34±0.14 [e]	69.80±0.15 [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- Glutathione AChE- Acetyl cholinesterase

3.5. Oxidative stress enzyme response in the liver of *Oreochromis niloticus* exposed to varying concentration of *Indigofera tictoria* aqueous extract is shown in Table 5

Significant increase (p>0.05) in the activity of all the enzymes in the liver as the concentration of *Indigofera tinctoria* aqueous extract was increased. In the treated group the ALT activity was elevated above the control, 2.81±0.01 IU/L in all the concentration. However, in fish exposed to the highest concentration 67 mg/l, the elevation was 80.28% 14.25±0.03 UI/L above the control (2.81±0.01 IU/L). *Indigofera tinctoria* aqueous extract caused an increase in CAT activity with the lowest value, 3.77±0.01 IU/L recorded at 13 mg/l and the highest 10.54±0.02 IU/L at 67 mg/l. SOD activity was elevated from 30.55±0.07IU/L in the control to 91.65±0.20 at 67 mg/l of *Indigofera tinctoria* aqueous extract. LDH activity was excited by 8.33%, and 26.67% in fish exposed to 13 mg/l and 67 mg/l aqueous extract respectively, above the control, 178.76±0.38 IU/L. GPX activity in the liver was elevated in all the exposed concentration and the highest 246.56±0.54 was observed in 67 mg/l, above the control, 204.27±0.44 IU/L. Significant increase in GSH was observed in all concentrations with a 15.29% at 67 mg/l above the control. Elevation was also recorded for AChE with 48.73% excitation at 67 mg/l above the control, 34.61±0.08 IU/L (Table 5).

Table 5: Biochemical indices in the liver of *Oreochromis niloticus* during 21 days exposure to *Indigofera tinctoria* aqueous extract

		Concentrations mg L^{-1}				
Treatments	**Control**	**13**	**27**	**40**	**53**	**67**
CAT	2.93±0.01 [a]	3.77±0.01 [b]	4.39±0.01 [c]	4.39±0.01 [c]	8.79±0.02 [d]	10.54±0.02 [e]
LDH	178.76±0.38 [a]	195.01±0.42 [b]	195.01±0.42 [b]	203.14±0.44 [c]	211.27±0.46 [d]	243.77±0.53 [e]
SOD	30.55±0.07 [a]	48.01±0.10 [b]	56.74±0.12 [c]	65.46±0.14 [d]	65.46±0.14 [d]	91.65±0.20 [e]
ALT	2.81±0.01 [a]	3.81±0.01 [b]	4.01±0.01 [c]	5.02±0.01 [d]	8.43±0.01 [e]	14.25±0.03 [f]
GPX	204.27±0.44 [a]	245.27±0.53 [a]	246.13±0.53 [a]	246.34±0.54 [a]	246.56±0.54 [a]	246.56±0.54 [a]
GSH	17.73±0.04 [a]	17.94±0.04 [b]	18.15±0.04 [c]	19.43±0.04 [d]	19.43±0.04 [d]	20.93±0.05 [e]
AChE	34.61±0.08 [a]	61.15±0.13 [b]	64.03±0.14 [c]	64.61±0.14 [d]	51.34±0.11 [e]	67.50±0.15 [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- Glutathione AChE- Acetylcholinesterase

3.6. Discussion

Variations in the physiological status of fish are determined by changes in water quality parameters such as temperature, pH, and dissolved oxygen [12, 13]. The concentrations of dissolved oxygen, pH, and temperature observed here match those reported by Mbah *et al.* [14]. The test solutions' physicochemical parameters revealed that the water temperature and pH varied slightly. There were some differences in dissolved oxygen, conductivity, and total dissolved solids. The concentration of dissolved oxygen decreased as the concentration increased, resulting in the death of the test fish. This is consistent with the findings of Idowu *et al.* [15] who found that when *Oreochromis niloticus* was exposed to *Kigeliaafricana* extract, dissolved oxygen in the water decreased. Akpa *et al.* [16] reported a decrease in dissolved oxygen with increasing concentration when *Tilapia zillli* was treated with *Tephrosia vogelii* leaf extract.

Under stressful conditions, such as exposure to pollutants, and harmful substances, haematological exams are frequently performed to establish health status and detect physiological abnormalities. Red blood cells, packed cell volume, and haemoglobin levels all decreased significantly. Etim *et al.* [17] found that the PCV level of *Oreochromis niloticus* fish exposed to *Indigofera tinctoria* (acute and chronic concentrations) was lower than the reference value for healthy fish. This could be owing to the poisonous potential of *Indigofera tinctoria* aqueous extract on the blood of *O. niloticus* as the concentration of the extract rises. This is consistent with Adakole [18] who discovered a similar tendency in fish exposed to metal finishing effluents. Furthermore, the lower haemoglobin level in the exposed fish compared to the control indicates that the fish's ability to give appropriate oxygen to the tissues is greatly diminished, resulting in a drop in physical activity [19]. Omoniyi *et al.* and Adewoye [20, 21] who subjected *Clarias gariepinus* juveniles to sublethal quantities of *Nicotiana tobaccum* and *Tephrosia Vogeli* extract, found a substantial drop in haemoglobin (Hb) concentration. The decrease in haemoglobin is due to a decrease in cellular iron, which causes a decrease in blood oxygen uptake capacity and eventually induces erythropoiesis, the state of anaemia. The number of white blood cells (WBCs) in *Oreochromis niloticus* treated to *Indigofera tinctoria* aqueous extract exhibited a substantial variation. The increasing trend of WBC could be attributed to the extract's immune-defense role against the effect of the extract's toxic potential, which is consistent with studies by Ayuba and Ofojekwu [22] on the toxicity of *Datura innoxia* (datura) to *Clarias gariepinus* fingerlings.

Increased ALT activity in the livers of exposed fish indicates hepatic cell destruction, which leads to their leaking into the

circulation [23]. Increased ALT activity in fish exposed to *Indigofera tinctoria* could be due to cholestasis and/or parenchymatous precursors linked to bile duct constriction at higher doses and full hepatocellular injury at moderate exposures. These findings coincide with Al-Attar [24] who showed an increase in ALT and ALP in *Oreochromis niloticus* after exposure to cadmium but contradict Das and Mukharjee [25], who identified a decrease in ALP activity in *Labeo rohita* after exposure to cypermetrin. The discrepancy in results may be related to the species of fish, toxicant used and duration of exposure. Lactate dehydrogenase (LDH) is an important enzyme often used as a biomarker of tissue breakdown and/or damage. The result of this study showed a significant increase in LDH in the liver, kidneys, and gills of *Oreochromis niloticus*, indicating that the tissues were damaged by the increasing concentration of *Indigofera tinctora*. This is consistent with the work of Audu *et al.* [26] who examined the effect of a differentially concentrated *Cannabis staiva* leaf extract on biochemical changes and the degree of change in the gills, liver, and serum of *Cyprinus carpio* during the 56-day experiment. This could be attributed to the assertion that toxins cause disturbances in the physiological state of animals that unintentionally affect enzyme activities, resulting in an increase or inhibition of enzyme activities [27]. Sunmonu and Oloyode [28] observed a similar trend in LDH activities in serum and gills of *C. gariepinus* fingerlings upon increasing sub lethal concentrations of crude oil compared to control. The increase in LDH observed in all experimental groups in this study may be due to necrosis of liver hepatocytes [29] and not necessarily to an increase in anaerobic carbohydrate metabolism [27]. Exposure to environmental pollutants can induce oxidative stress in an organism [30, 31, 32].

The catalase activity (CAT) determined in this study indicated some changes in liver, gill, kidney and muscle of *Oreochromis niloticus* exposed to *Indigofera tinctoria*. CAT is a sensitive antioxidant and its protein function can be affected by the products of lipid peroxidation, modulating the level of enzymatic activity [33]. The result obtained in the study is in agreement with the result obtained by Ibrahim [34], who found a significant increase in CAT activity in *Oreochromis niloticus* exposed to malathion for 30 days. The observed increase could be due to the animal scavenging H2O2, mainly due to the increased production of free radicals by *Indigofera tinctoria*. Nwani *et al.* [35] reported a significant increase in CAT activity of *Clarias gariepinus* exposed to *Psychotria microphylla* leaf extract for 15 days and stated that the significant increase observed shows that the leaf extract causes oxidative stress in the exposed fish, which is consistent with the result of this study. Superoxide dismutase (SOD) plays an important role in cellular antioxidant defence mechanism. The significant increase in SOD activity observed in this study in *Oreochromis niloticus* exposed to different concentrations of *Indigofera tinctoria* in liver, gills and kidneys is in agreement with the work of Ekeh *et al.* [36] who reported a significant increase in SOD activity of *Clarias gariepinus* exposed to sub-lethal concentration of potassium dichromate. The increase in SOD activity may indicate that O2 formation is occurring in the organism's tissues. Variations in SOD activity were also reported by Oruc [37] who exposed *Oreochromis niloticus* to chemical pollutant. The initial increase in SOD activity and gradual decrease at the 30th of 60 days of exposure is in contrast to the results of this study. This discrepancy could be due to the exposure period and the toxin used. The altered SOD activities observed in this study could be due to cellular oxidative stress caused by exposure to *Indigofera tinctoria*. Similar findings were made on the effects of pesticides on other fishes *Carassius auratus* exposed to Round Up [38] *Oreochromis niloticus* exposed to chlorpyrifos [37] *Cyprinus carpio* exposed to terbutyrin [39]. GSH is a low molecular weight sulfhydyl compound that acts as a cellular reducing and protective reagent against a wide range of pollutants through the SH-group [40]. It directly acts as a scavenger of oxyradical and also as an antioxidant enzyme-substrate [41]. In this study, a significant increase in GSH was observed in the tissue specimen of *Oreochromis niloticus* is in contrast with the work of Ozoagudike and Bawa-Allah [42] who reported a decrease in GSH activity in *Clarias gariepinus* exposed to a sub-acute concentration of *Carica papaya* seed and *Anacardium occidentale* bark for 28 days. Mowuogwu and George [43] reported increased GSH level in *Clarias gariepinus* exposed to *Hibiscus sabdariffa* extract for 21 days and Lima *et al.* [44] indicated that increased GSH levels in *Oreochromis niloticus* exposed to a contaminated effluent appear to be an antioxidant adaptation to chronic exposure. An increase in the GSH activity in the various tissues observed could also be attributed to phytochemical constituents of the aqueous extract used which possess antioxidant activity thereby having a sparing effect on GSH.

4. Conclusion and Recommendation

The present findings have established that sub-acute concentrations of *Indigofera tinctoria* induce oxidative stress and hepatotoxicology in Nile tilapia and this gives an insight on the impact of *Indigofera tinctoria* effluents on fish (fin and shell fish) health as they are the sole inhabitant of the aquatic environment which receives these effluents. There is the need to make proper policy and laws that makes it mandatory for industries to treat their effluents effectively before discharging they are discharged into the environment. The effect of *Indigofera tinctoria* effluents on aquatic organisms can be monitored by oxidative stress and transaminase enzymes' activities as in this study.

5. Acknowledgement

The authors are grateful to the Department of Fisheries and Aquaculture Technology, Department of Biochemistry, Federal University of Technology Akure, for their financial, technical support during sampling.

6. 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.

References

1. Oladele AK, Gabriel LM, Ibanga UI. Proximate composition and selected heavy metals concentration of smoked catfish (*Clarias gariepinus*) and Tilapia (Oreochromis niloticus) around lake Chad. In: Proceedings of the 20th Annual Conference of the Fisheries Society of Nigeria (FISON); c2005. p. 400-401.
2. Omoregie E. Effect of petroleum in Nile tilapia and its helminthes infection Ph. D Thesis, University of Jos, Jos, Nigeria. 1995, 152pp.

3. Ogundele O, Alatise PS, Oyawoye SO, Awoku DT. Effect of Linear Alkyl Benzene Sulphonate (LAS) on the weight gain of *Clarias gariepinus*. In: proceedings of the 20th Annual Conference of the Fisheries Society of Nigeria (FISON) in Port Harcourt. 2005 Nov14th-18th, p. 397-398
4. Offem BO, Ayotunde EO, Ikpi GU. Toxicity and accumulation of lead in the muscle of Nile Tilapia Oreochromis niloticus (Linnaeus, 1758) from fish pond in Cross River State, Nigeria Journal of Field Aquatic Studies. 2005;2(1):1-7.
5. Fagbenro OA. Tilapia: Fish for thought. Inaugural Lecture Series 32. Delivered at Federal University of Technology, Akure; c2002. p. 77.
6. Perry NSL, Houghton PJ, Theobald A, Jenner P, Perry EK. *In vitro* inhibition of human erythrocyte acetyl cholinesterase by *Salvia lavandulaefolia* essential oil and constituent terpenes. J Pharmacy and Pharmacol. 2000;52:895-902.
7. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972;247:3170-3175.
8. Mahmoud H. New method for assessment of serum catalase activity. Indian Journal of science and technology. 2016;9(4):1.
9. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med. 1963;61:882-8.
10. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, *et al.* Selenium: biochemical role as a component of glutathione peroxidase. Science. 179(4073):588-90.
11. Reitman S, Frankel SA. Colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 1957(1):56-63.
12. Bridget SG, Fisher R. Temperature influences swimming speed, growth and larval duration in coral reef fish larval. Journal of Experimental Marine Biology and Ecology. 2004;299:115-132.
13. Byström P, Andersson J, Kiessling A, Eriksson LO. Size and Temperature Dependent Foraging Capacities and Metabolism. Consequences for Winter Starvation Mortality in Fish. Oikos. 2006;11:43-52.
14. Mbah CE, Alo MN, Ugah UI, Owolabi JO, Oluwatoyin OO, *et al.* Assessment of microbial quality of surface and underground water in thr Fedearl university, Ndufu-Alike, Ikwo. FUNAI Journal of Science and Technology. 2016;2(1):1-9.
15. Idowu AA, Popoola OC, Alani JO, Ipadeola A, Nwekoyo VE. Toxicity effect of *Kigelia Africana* aqueous extract on the haematology and histopathology of juvenile Nile tilapia (*Oreochromis niloticus*). Journal of Tropical Agriculture, Food, Environment and Extension. 2020;19(1):37-42.
16. Akpa LE, Ajima MNO, Audu BS, Labte SM. Effect of fish bean (*Tephrosia vogelii*) leaf extract exposed to freshwater cichlid fish (*Tilapia zilli*). Animal research International. 2010;7(3):1236-1241.
17. Etim L, Olele FN, Eriegha OJ. Blood characteristics of *Oreochromis niloticus* (Nile Tilapia) from Ona Lake, Asaba, Nigeria. African Journal of Environmental Pollution and Health. 2009;7(1):43-46.
18. Adakole JA. Changes in some haematological parameters of the African catfish (*Clarias gariepinus*) exposed to a metal finishing company effluent. *Indian* Journal of Science and Technology. 2012;5(4):2510-2514.
19. Kori-Siakpere O, Ogbe MG, Ikomi RB. Blood profile of the African catfish: *Clarias gariepinus* (Burchell, 1822) to sub-lethal concentrations of potassium permanganate. Scientific Research and Essay. 2009;4(5):457-466.
20. Omoniyi I, Agbon AO, Sodunke SA. Effect of lethal and sublethal concentrations of Tobacco *(Nicotiana tobaccum*) leaf dust extract on weight and haematological changes in *Clarias gariepinus* (Burchell). Journal of Applied Sciences and Environmental Management. 2002;6(2):37-4.
21. Adewoye SO. Haematological and biochemical changes in *Clarias gariepinus* exposed to *Tephrosia vogelii* extract. Advance in Applied Science Research. 2010;1(1):74-79.
22. Ayuba VO, Ofojekwu PC. Acute toxicity of the root extract of Jimson's Weed: *Datura innoxia* to the African catfish (*Clarias gariephinus*) fingerlings. Journal of Aquatic Science. 2002;17(2):131-133.
23. Mousa MMA, El-Ashram AMM, Hamed M. Effects of Neem leaf extract on freshwater fishes and zooplankton community. 8th International symposium on Tilapia in aquaculture. The Central Laboratory for Aquaculture Research, Cairo, Egypt; c2008.
24. Al-Attar AM. Biochemical effects of short-term cadmium exposure on the freshwater fish, Oreochromis niloticus. Journal of Biological Sciences. 2005;5(3):260-265.
25. Das BK, Mukherjee MD. Toxicity of Cypermethrin in *Labeo rohito* fingerlings: biochemical enzymatic and haematological consequences. *Comparative* Biochemistry and Physiology Part C: Toxicology and Pharmacology. 2003;134:109-121.
26. Audu BS, Ajima MN, Ofojekwu PC. Enzymatic and biochemical changes in common carp, *Cyprinus carpio* (L.) fingerlings exposed to crude leaf extract of *Cannabis sativa* (L.)", Asian Pacific Journal of Tropical Disease. 2015;5(2):07-115.
27. Kori-Siakpere O, Ikomi RBO, Ogbe MG. Variation in acid phosphatase and alkaline phosphatase activities in the plasma of the African catfish: *Clarias gariepinus* exposed to sublethal concentrations of potassium permanganate. Asian Journal of Experimental Biological Sciences. 2010;1(1):170-174.
28. Sunmonu TO, Oloyede OB. Changes in liver enzyme activity in African catfish *Clarias gariepinus* exposed to crude oil. Asian Fisheries Science. 2006;19:107-112.
29. Vasudevan DM, Sreekumari S, Kannan V. Textbook of biochemistry for medical students. 6th ed. New Delhi, India: Jaypee Brothers, Medical Publisher. c2010.
30. Doherty VF, Ogunkuade O, Kanife UC. Biomarkers of oxidative stress and heavy metal levels as indicators of environmental pollution in some selected fishes in Lagos, Nigeria. American Eurasian Journal of Agriculture and Environmental Science. 2010;7:359-365.
31. Farombi EO, Adewole OA, Ajimoko YR. Effect of butachlor on antioxidant enzyme status and lipid peroxidation in fresh water African Catfish, (*Clarias gariepinus*). International Journal of Environmental Research and Public Health. 2007;5:423-427.
32. Olagoke O. Lipid Peroxidation and Antioxidant Defense

Enzymes in *Clarias gariepinus* as Useful Biomark-ers for Monitoring Exposure to Polycyclic Aromatic Hydrocarbons. MSc. Theses, University of Lagos Nigeria; c2008 p. 70.
33. Bagnyukova TV, Chahrak OI, Lushchak VI. Coordinated response of goldfish antioxidant defenses to environmental stress. Aquatic Toxicology. 2006;78:325-331.
34. Ibrahim AT. Biochemical and histopathological response of *Oreochromis niloticus* to malathion hepatotoxicity. Journal of Royal Science. 2019;1(1):10-15.
35. Nwani CD, Nwadinigwe AO, Joshua PE, Onyeke CC, Ogbonna SU, *et al.* Hepatic antioxidant status and hematological parameters in African catfish *Clarias gariepinus* juvenile exposed to sublethal concentration of *Psychotria microphylla*. The Journal of Animal and Plant Science. 2016;26:275-281.
36. Ekeh, FN, Ekechukwu EN, Atama CI, Ezenwajiaku FI, Ohanu CM, *et al.* Oxidative stress responses of juvenile catfish, *Clarias gariepinus* exposed to potassium dichromate at sublethal concentration in south eastern Nigeria. African Journal of Aquatic Science. 2018;43(4):393-403.
37. Oruc EO. Oxidative stress, steroid hormone concentrations and acetylcholinesterase activity in *Oreochromis niloticus* exposed to chlorpyrifos. Pesticides Biochemistry and Physiology. 2010;96:160-166.
38. Lushchak OV, Kubrak OI, Storey JM, Storey KB, Lushchak VI. Low toxic herbicide roundup induces mild oxidative stress in goldfish tissues. Chemosphere. 2009;76(7):932-937.
39. Velisek J, Stara A, Kolarova J, Svobodova Z. Biochemical, physiological and morphological responses in common carp (*Cyprinus carpio* L.) after long-term exposure to terbutryn in real environmental concentration. Pesticide Biochemistry and Physiology. 2011;100(3):305-313.
40. Zhang JF, Shen H, Wang XR, Wu J, Xue Y. Effects of chronic exposure of 2,4-dichlorophenol on the antioxidant system in liver of freshwater fish *Carassius auratus*. *Chemosphere*. 2004;55(2):167-174.
41. Li ZH, Zlabek V, Grabic R, Li P, Machova J, *et al.* Effects of exposure to sublethal propiconazole on the antioxidant defense system and Na+-K+-ATPase activity in brain of rainbow trout, Oncorhynchus mykiss. Aquatic Toxicology. 2010;98(3):297-303.
42. Ozoagudike CM, Bawa-Allah K. Comparative assessment of the acute and subacute effects of some plant based ichthyotoxins on aquatic organisms: A case study of *Clarias gariepinus*. Third edition on Sustainable development goal 14. 2020;13(2s):36-45.
43. Mowuogwu FO, George BO. Comparative antioxidant activity of Hibiscus sabdariffa and ascorbic acid on ferrous sulohate induced oxidative stress in *Clarias gariepinus*. East and Central African Journal of Pharmaceutical Sciences. 2008;11:39-42.
44. Lima PL, Benassi JC, Pedrosa RC, Dal Magro J, Oliveira TB, *et al.* Time-course variations of DNA damage and biomarkers of oxidative stress in tilapia (*Oreochromis niloticus*) exposed to effluents from a Swine industry. Archives of Environmental Contamination and Toxicology. 2006;50:23-30.