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Original article

BIOCHEMICAL AND PHYSIOLOGICAL RESPONSES OF HETEROCLARIAS AND RAT FED ON CONTAMINATED HETEROCLARIAS TO DIESEL OIL-INDUCED STRESS

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ABSTRACT

Impacts of sub-lethal concentrations of water soluble fraction of diesel-oil (WSFD) on biochemical and physiological responses of hybrid catfish, Heteroclarias and rats fed on contaminated-Heteroclarias-were investigated. Heteroclarias was assessed in a static renewal bioassay for 28 days using varying concentrations (0.00, 15.63, 31.25, 62.50, 125.00, and 250.00 ppm) of WSFD, while rats were fed with diet compounded with WSFD-contaminated-Heteroclarias for 30 days. The WSFD-exposed fish and the WSFD-contaminated-Hetroclarias-fed rats were sacrificed, blood samples were collected and the gill and liver of the fish as well as the liver and kidney of the rat were removed for assay. In both animals, red blood cell, haemoglobin, packed cell volume, protein and glucose decreased, while the white blood cell increased compared to the control. Serum activities of alanine aminotransferase, aspartate aminotransferase and superoxide dismutase significantly increased in WSFD-exposed Heteroclarias and WSFD-contaminated-Heteroclarias-fed rat, while the enzymes' activities were inhibited in the gill and liver of the fish and liver and kidney of the rat. Lactate dehydrogenase activity decreased in the serum of both WSFD-exposed Heteroclarias and WSFD-contaminated-Heteroclarias-fed rat but increased in the gill and liver of WSFD-exposed Heteroclarias and liver and kidney of WSFD-contaminated-Heteroclarias-fed rat. There was a significant ($P < 0.05$) reduction in the specific growth rate of WSFD-exposed Heteroclarias and rat fed WSFD-contaminated-Heteroclarias as the concentrations increased. Therefore, toxic effects of diesel-oil on aquatic organisms should be monitored, so as to ensure safety, survival and sustainable exploitation of healthy fish as well as prevent risk of health disorder in fish consumers.

Keywords: Heteroclarias; Rat; Diesel-oil; Contaminated-Heteroclarias-diet; Toxicity; Public health

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INTRODUCTION

The Nigerian economy is highly dependent on oil and gas. Oil and gas exploration has not only significantly impinged on the integrity of the environment negatively but also contributes to the worsening global environment and public health related problems. In the Niger Delta region, oil spillage has been a major phenomenon and this has seriously exerted severe impact on the surrounding ecosystems (Wegwu and Akaninwor, 2006). In this region, rivers, lakes, groundwater, soil, plants and fisheries are adversely impacted by the exploration and exploitation of fossil fuel resources. The damage to the aquatic environment in this region does not only affects the quality of life and health of the people, but also the ability of the people to participate in meaningful economic development.

Diesel oil is one of the most commonly used automotive fuel in the world and among the different types of aquatic oil pollutants, it exhibits low water solubility. The water-soluble fraction of diesel oil (WSFD) and their derivatives contains a mixture of several toxic constituents like polycyclic aromatic hydrocarbons (PAHs), monoaromatic hydrocarbons and heavy metals (Rodrigues *et al*., 2010). Although, these constituents have low water solubility, they are nonetheless being accumulated and retained in the lipophilic tissues of aquatic organisms more than the alkanes, hence making diesel oil to be highly toxic. PAHs, like naphthalene, fluorine and phenanthrene have been reported to be among the major chemical components of diesel oil that pose the greatest hazard to aquatic organisms (Lee and Anderson, 2005, Simonato *et al*., 2008). Oil spills and leakages from oil wells and burst pipes have been implicated as the major cause of water contamination (Simonato *et al*., 2011). The exposure of fish to oil-contaminated water could cause varying degrees of biochemical and physiological effects that may in turn affect fish's health and ultimately that of human beings that depend on fish for protein and essential minerals like calcium, iron and phosphorous.

Reported damage of water soluble fraction of oil and their derivatives to fish include inactivation or alterations in enzyme activity (Livingstone, 2001, Adeyemi *et al*., 2009), DNA damage (Zhang *et al*., 2004), tissue architecture (Gabriel *et al*., 2007) and ultimate death leading to depletion of fish stocks. Besides the consumption of oil contaminated-fish, crude oil has been reported to be used in folkloric medicine for the treatment of various ailments in the Niger-Delta region (Orisakwe *et al*., 2000). In addition, the sucking of petrol to wash hands is also a common practice by the roadside automobile mechanics (Ita and Udofia, 2011). This, therefore, raises a serious public health concern and hence the understanding of the toxic potential of diesel oil to aquatic organisms and their consumers becomes imperative.

Heteroclarias is an inter-generic hybrid of two most popular African Clariid catfishes, *Clarias angullaris* and *Heterobranchus bidorsalis*. *C. angullaris* and *H. bidorsalis* are commercially grown freshwater fishes in Nigeria and are of great importance locally as they serve as delicacies among many tribes. In

aquaculture system, production of hybrid is usually undertaken in attempt to enhance faster growth, survival and improvement of generic traits. Heteroclarias has been found to be hardy and can survive adverse condition as much as its pure lines (*C. angullaris* and *H. bidorsalis*) as evident by its haematological profile (Diyaware *et al.*, 2013), thus informing its choice for this ecotoxicological study. There are many reports on the potential toxic effects of water soluble fraction of crude oil and their derivatives on fish but information on the effects of WSFD on hybrid catfish is scarse. The health of fish inhabiting a body of water is a function of the quality of water being inhabited by the fish. This study, therefore, sought to evaluate the effects of WSFD on some aspects of the physiology of hybrid catfish, Heteroclarias and that of diesel oil contaminated Heteroclarias-fed rat.

MATERIALS AND METHODS

Animal maintenance

Juvenile Heteroclarias (average weight: 11.09 $\pm$ 0.42 g and average length 9.04 $\pm$ 0.71 cm) of the same brood stock were obtained from a commercial fish hatchery in Ilorin ((10^{0}53'0"N, 4^0 1' 0"E), Kwara State, Nigeria and transported in plastic aquaria containing water from the hatchery to the laboratory. The fish were not fed prior to transportation and on arrival at the laboratory until the next day; so as to reduce to bearest minimum mortality due to stress.

In the laboratory, fish were held and acclimated to laboratory condition for 14 days in a 200-litre capacity tank containing borehole water and maintained at temperature ranging from 24^{o}C to 27^{o}Cusing a 300 Watt AZOO submersible thermometer with thermostat. Fish were fed twice daily at 9.00 am and 4.00 pm with commercial fish feed at 3% body weight. Water was changed every two days to reduce the risk of mortality due to accumulation and contamination of waste materials. Fish were not fed 24 hours before the commencement of the experiment. Physico-chemical parameters of the test media were monitored using standard methods (APHA and AWWA, 1998) and were found as follows: temperature 23.86 $\pm$ 1.25^{o}C,pH 6.92 $\pm$ 0.78, dissolved oxygen 6.58 $\pm$ 1.43 mg/l, conductivity 0.84 $\pm$ 0.35µ/s and biological oxygen demand 18.28 $\pm$ 2.10 mg/l and a 12 h light: 12h dark photoperiod was maintained. Albino rats (average weight:63.22 $\pm$ 5.77) were obtained from the Animal Holding Unit of the Department of Zoology, University of Ilorin, Kwara State, Nigeria and were also acclimated to the housing conditions for 14 days prior to commencement of experiment. Animals were housed at ambient temperature 22.86 $\pm$ 3.28^{0}C and a 12 h light: 12 h dark cycle. The rats were fed *ad libitum* during this period.

Preparation of water soluble fraction of diesel oil

Diesel oil was obtained from a gas station in Ilorin, Kwara State. The water soluble fraction of diesel oil (WSFD) was prepared following the procedure proposed by Nicodem *et al* (1998). This procedure was employed to simulate the naturally occurring leakage/spillage of fuel in aquatic environment. The diesel oil was diluted with deionized water in a glass container in a proportion of 1 part (1-litre) of diesel oil to 4 parts (4-litre) of deionised water. The deionised water-

diesel mixture was subsequently stirred slowly and left under sunlight for 6 hours so as to enhance the dissolution of the soluble components of the fuel in water. The mixture was then poured into different separating funnels and allowed to stand for 24 hours so as to obtain a clear oil-water interphase. The lower aqueous layer containing the WSFD was separated from the organic (insoluble) layer by decantation and stored in containers for use in the exposure assay. This process was severally repeated for each mixture until sufficient quantity of the WSFD was obtained to carry out the study, while the insoluble layer was discarded.

Experimental protocol

Based on the result of an earlier presumptive test, three replicates of ten acclimated fishes of equal weight were exposed to ¼ each of the acute concentrations i.e. 0.0 (Control), 15.63, 31.25, 62.50, 125.00 and 250.00 ppm WSFD solutions. The weight of the fishes in each concentration was measured using Ohaun compact digital weighing balance (Mettler Instrument) and recorded as initial weight (W_1). Each of the WSFD media was renewed every 48 hours. The experiment was carried out using a semi-static renewal method to keep the toxicant concentration constant (Reish and Oshida, 1987). Physico-chemical parameters (BOD, COD, temperature, conductivity, dissolved oxygen and pH) of the test media were monitored using the method of APHA (1995). At the end of the experiment, the weight of the fishes were taken and recorded as final weight (W_2). Growth was expressed as absolute weight gain, percentage weight gain and specific growth rate. Specific growth rate (SGR) was estimated using the formula: SGR = 100 x ln W_2 – ln W_1/t where W_1 and W_2 represent the initial weight and final weight of fish in each concentration, respectively, and t is the test duration in days.

At the end of the 28-day exposure period, fish were randomly collected from each concentration for blood sampling and the gills and liver were removed for biochemical analysis. The body carcass of the exposed fish in each treatment were then used in the formulation of diet for rats. Fish were oven dried at 40^0C and used as protein source (25%) which was thoroughly mixed with 52% corn starch, 4% oil, 10% sucrose and 5% vitamin/mineral. Rats were randomly grouped into six and assigned into different cages with each group containing ten rats per cage. Those in group 1 served as the control and were fed with the control diet, which was formulated with fishes raised in bore-hole water (i.e. those not exposed). Rats in group 2 to 6 were fed with diet formulated with fishes exposed to the different concentrations of WSFD (i.e. WSFD-contaminated Heteroclarias) for 30 days. The rats in each group were provided with clean water throughout the experiment and were monitored for physical and behavioural changes. Body weights were taken on weekly basis for growth assessment using the growth evaluation formulae earlier described.

Haematological and biochemical assays

At the end of the experiment, control and WSFD exposed-fish were removed as well as the rat fed WSFD exposed-fish. Blood samples were taken from the caudal vein of the fish and tail of rat, respectively using heparinized plastic syringes.

Immediately after sampling, part of the blood, approximately, 0.5 ml aliquots for fish and 0.7 ml aliquots for rat were centrifuged at 350 g for 5 min using refrigerated centrifuge in order to separate serum for biochemical analyses. Animals were then subjected to humane killing to remove the gill, liver and kidney.

RBC and WBC were determined using an improved Neubauer haemocytometer viewed under a light microscope at x400 magnification; after the blood had been diluted with Dacie's fluid (Dacie and Lewis, 2001) and stained with brilliant cresyl blue. For RBC count a dilution of 1: 2000 was used, while for WBC count a dilution of 1: 1, 500 was used. Haemoglobin (HB) concentration was estimated by cyanomethalomoglobin method (Blaxhall and Daisley, 1973). Haematocrit (PCV) was determined by the microhaematocrit method (Nelson and Morris 1989). Erythrocytes indices (mean cell volume (MCV), mean cell haemoglobin (MCH) and mean cell haemoglobin concentration (MCHC) was estimated by Dacie and Lewis' (2001) formulae.

One gramme each of the gill, liver and kidney was weighed and homogenized with 5ml of 0.25M sucrose solution in ice-cold condition. The homogenate of each tissue was divided into two portions; first one, approximately, 150-200 µg/l were centrifuged at 14,700 g and 4 ^{o}C for 20 min to obtain the supernatant. This was used to determine the activities of ALT, AST, SOD and LDH. Serum and tissue activities of ALT and AST were determined by the procedure of Reitman and Frankel as described by Ochei and Kolhatkar (2005). SOD was assayed by the method of Misra and Fridovish, (1972), while the procedure of DGKC (1970) was used in LDH assay. The values of RBC, HB and PCV obtained were used to calculate MCV, MCH and MCHC using the formulae given by Dacie and Lewis (2001). Glucose was determined using Trinder (1969) method, while protein level was estimated according to the method of Lowry *et al*. (1951) using bovine serum albumin (BSA) as standard.

Data Analysis

Data obtained were subjected to parametric (One way ANOVA) or non-parametric (Kruskal-Wallis) analysis depending on the distribution and homogeneity of the data. Tukey's multiple range test was used to determine significant differences and values were considered significant when $P<0.05$.

RESULTS

Haematological and metabolic parameters of Heteroclarias exposed to varying concentrations of WSFD for 28 days are shown in Table 1. RBC, HB, PCV, MCV, MCH and MCHC significantly decreased ($P < 0.05$) as the concentration of WSFD increased compared to the control. WBC significantly increased ($P < 0.05$) with increase in concentrations of WSFD. In contaminated Heteroclarias-fed rat. RBC, HB and PCV were significantly reduced ($P < 0.05$) compared to the control (Table 2). WBC significantly increased with increase in concentration, with the highest value recorded at 250.00 ppm, while the lowest value (5.60 ± 5.77 ppm) was recorded in the control. All the haematological indices (MCV, MCH and MCHC) varied insignificantly from the control. Protein and glucose levels in the blood of fish and contaminated-Heteroclarias fed rat in all treatments decreased significantly compared to the values recorded for control (Tables 1 and 2).

The enzyme activities in the blood, gill and liver of Heteroclarias exposed to different concentrations of WSFD for 28 days are shown in Tables 3-5. ALT and AST increased significantly in the blood (Table 3) but decreased in in the gill (Table 4) and liver (Table 5) compared to their respective control. LDH activity was significantly decreased in the blood but increased in the gill and liver of fish with increase in concentration of WSFD, while the activities of SOD significantly increased in all the tissues than the control, though values were not significantly different in the gill and liver of fish in all the treatments. In rat fed contaminated-Heteroclarias diet in different treatments, ALT and AST activities in the blood serum (Table 6) followed an increasing trend similar to the situation in the gill and liver of WSFD exposed-fish, but decreased in the kidney (Table 7) and liver (Table 8) compared to their respective control. LDH activity was significantly inhibited in the blood, while its activity was enhanced in the kidney and liver. However, SOD activity was significantly elevated in the blood, while its activity in the kidney and liver showed no significant difference from the control.

The specific growth rate of WSFD-exposed fish and WSFD contaminated-Heteroclarias fed rat significantly reduced as the concentration of WSFD increased compared to the control.

Table 1: Haematological and metabolic parameters of Heteroclarias exposed to different concentrations of WSFD for 28 days

Parameter	Concentration (ppm)					
	0.00	15.63	31.25	62.50	125.00	250.00
RBC ($x10^{12}$/l)	3.80 ± 5.77^{e}	3.65 ± 2.89^{d}	3.38 ± 5.77^{c}	3.34 ± 0.00^{b}	3.34 ± 1.12b	2.99 ± 5.77^{a}
HB (g/dl)	8.80 ± 5.77^{e}	8.60 ± 5.77^{d}	8.10 ± 5.77^{c}	8.00 ± 5.77^{c}	7.80 ± 0.00^{b}	7.60 ± 5.77^{a}
PCV (%)	26.50 ± 0.58^{e}	25.70 ± 0.58^{d}	25.00 ± 0.58^{c}	24.00 ± 0.58^{c}	22.00 ± 0.58^{b}	21.00 ± 0.58^{a}
MCV (µl)	83.00 ± 0.58^{d}	82.00 ± 0.67^{d}	79.00 ± 0.53^{c}	78.00 ± 0.81^{c}	74.00 ± 0.68^{b}	72.00 ± 0.55^{a}
MCH (pg)	30.00 ± 0.58^{d}	29.00 ± 1.12^{c}	28.00 ± 0.58^{b}	27.09 ± 1.73^{b}	27.00 ± 0.58^{b}	22.00 ± 0.58^{a}
MCHC (g/dl)	34.00 ± 1.15^{c}	30.00 ± 0.58^{b}	31.00 ± 0.58^{b}	29.00 ± 0.45^{a}	30.00 ± 0.58^{b}	28.63 ± 0.68^{a}
WBC ($x10^{9}$/l)	2.10 ± 5.77^{a}	2.00 ± 0.00^{a}	2.50 ± 5.77^{b}	2.89 ± 5.77^{c}	3.00 ± 0.00^{d}	3.40 ± 0.12^{e}
Protein (g/l)	102.00 ± 0.58^{f}	94.00 ± 0.58^{e}	87.00 ± 0.58^{d}	82.00 ± 0.58^{c}	74.00 ± 0.58^{b}	70.03 ± 0.58^{a}
Glucose (Mmol)	7.60 ± 0.12^{e}	6.08 ± 5.77^{d}	5.40 ± 0.12^{c}	$4.30 \pm +5.77^{b}$	4.60 ± 0.00^{b}	3.25 ± 5.77^{a}

Values are means ± SD of 3 replicates. Means along the same row with different superscripts are significantly (P<0.05) different. WSFD = Water soluble fraction of diesel oil, RBC = Red blood cell, HB = Haemoglobin, PCV = Packed cell volume, MCV = Mean cell volume, MCH = Mean cell haemoglobin, MCHC = Mean cell haemoglobin concentration, WBC= White blood cell

Table 2: Haematological and metabolic parameters in WSFD contaminated Heteroclarias-fed rat for 30 days

Parameter	Concentration (ppm)					
	0.00	15.63	31.25	62.50	125.00	250.00
RBC (x10^{12}/l)	5.43±1.73^{e}	5.35±5.77^{d}	5.02±5.77^{c}	5.00±0.00^{c}	4.71±0.00^{d}	4.21±5.77^{a}
HB (g/dl)	17.30±0.17^{e}	16.70±5.77^{d}	16.00±0.00^{c}	15.70±0.12^{b}	14.70±0.12^{a}	14.30±0.17^{a}
PCV (%)	52.00±1.15^{d}	50.00±0.58^{c}	48.00±0.58^{b}	47.00±0.58^{b}	44.00±173^{a}	43.00±0.58^{a}
MCV (µl)	96.00±0.57^{a}	94.00±1.15^{a}	96.00±0.58^{a}	95.00±0.58^{a}	93.00±1.73^{a}	93.00±0.58^{a}
MCH (pg)	31.00±0.58^{a}	31.00±0.58^{a}	32.00±0.58^{a}	31.00±0.58^{a}	32.00±0.58^{a}	32.00±0.58^{a}
MCHC (g/dl)	33.00±0.00^{a}	33.00±0.58^{a}	33.00±0.58^{a}	33.00±0.00^{a}	33.00±0.00^{a}	33.00±0.00^{a}
WBC (x10^{9}/l)	5.60±5.77^{a}	6.40±5.77^{b}	6.40±0.17^{b}	8.60±5.77^{c}	9.30±5.77^{c}	10.40±5.12^{d}
Protein (g/l)	82.36±8.07^{f}	80.24±6.87^{e}	78.42±6.31^{d}	75.28±7.13^{c}	72.09±5.94^{b}	68.95±4.99^{a}
Glucose (Mmol)	6.90±5.77^{e}	5.40±5.77^{d}	4.40±5.77^{c}	4.40±5.77^{c}	3.90±5.77^{b}	3.20±5.77^{a}

Values are means ± SD of 3 replicates. Means along the same row with different superscripts are significantly (P<0.05) different. WSFD = Water soluble fraction of diesel oil, RBC = Red blood cell, HB = Haemoglobin, PCV = Packed cell volume, MCV = Mean cell volume, MCH = Mean cell haemoglobin, MCHC = Mean cell haemoglobin concentration, WBC= White blood cell

Table 3: Enzyme activities (unit/g) in the blood of Heteroclarias exposed to different concentrations of WSFD for 28 days

Enzyme (unit/g)			Concentration (ppm)					
			0.00	15.63	31.25	62.50	125.00	250.00
A	L	T	0.21±0.01^{a}	0.32±0.01^{b}	0.32±0.01^{b}	0.41±0.01^{c}	0.42±0.01^{c}	0.48±0.01^{d}
A	S	T	0.21±0.01^{a}	0.26±0.01^{b}	0.27±0.01^{b}	0.31±0.01^{c}	0.39±0.01^{d}	0.39±0.01^{d}
L	D	H	0.36±0.02^{e}	0.32±0.01^{d}	0.30±0.01^{c}	0.29±0.02^{b}	0.28±0.01^{b}	0.26±0.00^{a}
S	O	D	1.65±0.02^{a}	2.40±0.02^{b}	2.46±0.02^{c}	2.54±0.01^{d}	2.20±0.01^{b}	1.85±0.03^{a}

Values are means ± SD of three replicates. Means along the same row with different superscripts are significantly different (P < 0.05).WSFD = Water soluble fraction of diesel oil, ALT = Alanine aminotransferase, AST= Aspartate aminotransferase, LDH = Lactate dehydrogenase, SOD = Superoxide dismutase

Table 4: Enzyme activities (unit/g) in the gill of Heteroclarias exposed to different concentrations of WSFD for 28 days

Enzyme (unit/g)			Concentration (ppm)					
			0.00	15.63	31.25	62.50	125.00	250.00
A	L	T	1.50±0.06^{e}	1.25±0.01^{d}	1.15±0.01^{c}	1.15±0.01^{c}	1.02±0.02^{b}	0.65±0.01^{a}
A	S	T	1.65±0.03^{d}	1.40±0.01^{c}	1.30±0.02^{c}	0.82±0.01^{a}	1.01±0.01^{b}	0.75±0.01^{a}
L	D	H	0.60±0.06^{a}	0.67±0.01^{b}	0.80±0.04^{c}	0.85±0.02^{d}	0.92±0.02^{e}	1.02±0.01^{f}
S	O	D	0.00±0.00^{a}	0.02±0.00^{b}	0.03±0.01^{c}	0.02±0.00^{b}	0.02±0.00^{b}	0.01±0.01^{a}

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Values are means ± SD of three replicates. Means along the same row with different superscripts are significantly different (P < 0.05).WSFD = Water soluble fraction of diesel oil,ALT = Alanine aminotransferase, AST= Aspartate aminotransferase, LDH = Lactate dehydrogenase, SOD = Superoxide dismutase

Table 5: Enzyme activities (unit/g) in the liver of Heteroclarias exposed to different concentrations of WSFD for 28 days

			Concentration (ppm)					
Enzyme (unit/g)			0.00	15.63	31.25	62.50	125.00	250.00
A	L	T	24.05±0.03^{e}	23.20±0.12^{d}	22.50±0.06^{c}	22.50±0.06^{c}	19.15±0.01^{b}	9.80±0.03^{a}
A	S	T	23.10±0.06^{e}	22.62±0.01^{d}	17.02±0.01^{c}	16.80±0.12^{b}	16.40±0.06^{b}	12.25±0.03^{a}
L	D	H	1.65±0.03^{a}	1.68±0.01^{b}	1.75±0.01^{c}	2.10±0.02^{d}	2.16±0.03^{e}	2.60±0.06^{f}
S	O	D	0.01±0.00^{a}	0.03±0.01^{b}	0.03±0.00^{b}	0.03±0.01^{b}	0.03±0.00^{b}	0.04±0.01^{b}

Values are means ± SD of three replicates. Means along the same row with different superscripts are significantly different ($P < 0.05$).WSFD = Water soluble fraction of diesel oil, ALT = Alanine aminotransferase, AST= Aspartate aminotransferase, LDH = Lactate dehydrogenase, SOD = Superoxide dismutase

Table 6: Enzyme activities (unit/g) in the blood of WSFD contaminated Heteroclarias-fed rat

			Concentration (ppm)					
Enzyme (unit/g)			0.00	15.63	31.25	62.50	125.00	250.00
A	L	T	0.27±0.01^{a}	0.31±0.01^{b}	0.31±0.02^{b}	0.59±0.04^{c}	0.67±0.02^{d}	0.72±0.03^{e}
A	S	T	0.19±0.00^{a}	0.23±0.01^{b}	0.41±0.02^{c}	0.47±0.02^{d}	0.50±0.00^{e}	0.59±0.05^{f}
L	D	H	2.75±0.56^{f}	2.50±0.01^{e}	2.35±0.02^{d}	2.20±0.01^{c}	2.05±0.02^{b}	1.80±0.03^{a}
S	O	D	1.65±0.00^{a}	1.80±0.01^{b}	2.05±0.02^{c}	2.26±0.04^{d}	3.35±0.06^{e}	3.30±0.01^{f}

Values are means ± SD of three replicates. Means along the same row with different superscripts are significantly different ($P < 0.05$).WSFD = Water soluble fraction of diesel oil, ALT = Alanine aminotransferase, AST= Aspartate aminotransferase, LDH = Lactate dehydrogenase, SOD = Superoxide dismutase

Table 7: Enzyme activities (unit/g) in the kidney of WSFD contaminated Heteroclarias-fed rat

			Concentration (ppm)					
Enzyme (unit/g)			0.00	15.63	31.25	62.50	125.00	250.00
A	L	T	16.40±0.03^{a}	12.90±0.06^{b}	12.06±0.03^{b}	11.27±0.01^{c}	10.75±0.03^{d}	10.20±0.12^{e}
A	S	T	10.12±0.01^{c}	9.45±0.03^{b}	9.39±0.02^{b}	9.39±0.02^{b}	9.14±0.02^{b}	8.85±0.03^{a}
L	D	H	24.07±0.05^{a}	28.90±0.06^{c}	26.05±0.03^{b}	31.05±0.03^{d}	28.90±0.02^{c}	31.55±0.03^{d}
S	O	D	0.03±0.06^{a}	0.03±0.03^{a}	0.04±0.01^{a}	0.05±0.02^{b}	0.06±0.02^{b}	0.06±0.01^{b}

Values are means ± SD of three replicates. Means along the same row with different superscripts are significantly different ($P < 0.05$).WSFD = Water soluble fraction of diesel oil, ALT = Alanine aminotransferase, AST= Aspartate aminotransferase, LDH = Lactate dehydrogenase, SOD = Superoxide dismutase

Table 8: Enzyme activities (unit/g) in the liver of WSFD contaminated Heteroclarias-fed rat

			Concentration (ppm)					
Enzyme (unit/g)			0.00	15.63	31.25	62.50	125.00	250.00
A	L	T	14.25±0.03^{f}	13.69±0.02^{e}	13.08±0.01^{d}	12.40±0.03^{c}	11.20±0.03^{b}	9.68±0.05^{a}
A	S	T	15.85±0.06^{d}	15.27±0.03^{d}	14.96±0.04^{c}	14.20±0.11^{b}	12.45±0.03^{a}	12.37±0.01^{a}
L	D	H	19.05±0.03^{a}	22.15±0.03^{b}	23.15±0.06^{b}	31.95±0.03^{c}	33.60±0.03^{d}	34.07±0.01^{e}
S	O	D	0.03±0.00^{b}	0.03±0.01^{b}	0.04±0.01^{b}	0.03±0.00^{b}	0.02±0.01^{a}	0.02±0.01^{a}

Values are means ± SD of three replicates. Means along the same row with different superscripts are significantly different ($P < 0.05$).WSFD = Water soluble fraction of diesel oil, ALT = Alanine aminotransferase, AST= Aspartate aminotransferase, LDH = Lactate dehydrogenase, SOD = Superoxide dismutase

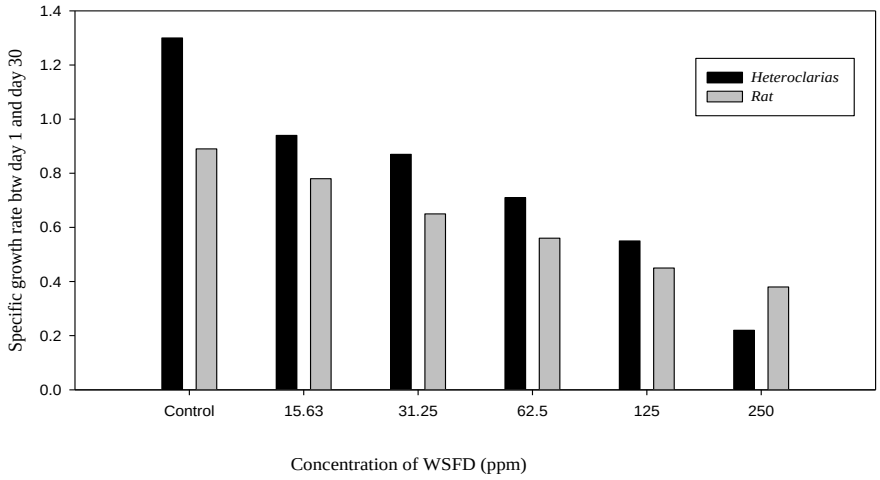

Fig. 1: Specific growth rate of *Heteroclarias* exposed to different concentrations of WSFD and rat fed WSFD-contaminated *Heteroclarias*

DISCUSSION

The present results showed that exposure of fish to diesel oil and consumption of diesel oil-contaminated fish may elicit severe impacts on various aspects of fish physiology and that of its consumers. Tissues/organs such as the blood, gill, liver and kidney are known to be the primary target sites of pollutants action in vertebrates where biochemical and pathological alterations can easily be detected. Hence, these tissues/organs serve as a good medium for ecotoxicological evaluations.

Haematological parameters constitute one of the first detectable stress responses caused by environmental perturbations (Davidson *et al*., 1993) and are sensitive indicators that can provide information about the health status of aquatic organisms (Rauf, 2015). In this study, the significant decrease in the

values of RBC, HB and PCV in both WSFD exposed-fish and WSFD contaminated Heteroclarias-fed rat are indicative of anaemia and could be attributed to haemolysis. This is further corroborated by a similar decrease in MCV, MCH and MCHC suggesting that the observed decrease in these parameters could actually be due to the haemolytic effect of the toxicant. The reduction in these parameters is also an indication of impaired oxygen delivery to tissues in both animals. Bloom and Brandt (2008) reported that haemolysis could cause oxygen impairment, thus interfering with the transport system in animals.

Petroleum hydrocarbons have been known to impair oxygen transport system by altering the membrane permeability of RBCs (Duarte *et al*., 2010). Similar findings in fish exposed to water soluble fractions of crude oil (Simonato *et al*., 2008, 2013) and in rat fed diesel oil-contaminated diet (Sunmonu and Oloyede, 2007, Achuba and Ogwumu, 2014) have been reported. The changes in MCV, MCH and MCHC that accompanied that of RBC, HB and PCV is understandable because the latter indices are normally calculated on the basis of the former. Increase in WBCs (leukocytosis) is a usual response of animals to stress condition. The increased WBCs (Tables 1 and 2) shows that WSFD triggered the activation of the immune system to defend the body against toxicity effect that could predispose the animals to secondary infection. Diesel oil can damage the structural integrity of fish (Kakkar*et al*. 2011) and rat (Achuba and Ogwumu, 2014) tissues and this could in turn elicit an enhanced WBC counts (Kalender *et al*., 2009). Earlier studies in our laboratory yet unpublished had revealed that the gill and liver architectures in WSFD-exposed Heteroclarias and that of the liver and kidney in contaminated Heteroclarias-fed rat exhibited degenerative changes, thus further corroborating the earlier observation of Kalender*et al* (2009) that damage to tissues can enhance the level of WBCs. The increase in WBC counts corresponds to the observations made on *Sarotherodon melanotheron* (Obemeata *et al*., 2012) and rat, *Rattus rattus* (Dede and Kagbo, 2002) following exposure to diesel oil.

The significant increase in serum transaminases' (ALT and AST) activities observed in this study may be due to structural damage to the gill and liver of WSFD-exposed fish and the liver and kidney of contaminated Heteroclarias-fed rat. This damage might have arisen from the alteration of the permeability of membranes; which resulted in the leakage of ALT and AST from these tissues/organs into the serum as evidenced by the significant reduction of the enzymes in the gill and liver of fish (Tables 4 and 5) or liver and kidney of rat (Tables 7 and 8) with increasing concentration of WSFD. This is likely to be a survival strategy to detoxify the toxicant. These observations agree with the findings of Adeyemi *et al* (2009) on *Clarias gariepinus* exposed to PAHs, Santos *et al* (2013) on *Prochilodus vimboides* exposed to diesel oil and Patrick-Iwuanyanwu (2011), Achuba and Ogwumu (2014) and Ikanone *et al* (2017) on rat fed diesel-oil and its derivative contaminated diet, respectively.

Glucose level in animals is a very useful biomarker for diagnosis of liver functions and that of muscles (Chen *et al*., 2004).

The low serum glucose level recorded in the experimental animals, which decreased depending on the concentration of WSFD might have resulted from hypoglycaemia. This hypoglycaemic condition is correlated with the inhibitory activities of the transaminase enzymes noticed in the gills and liver of exposed fish (Tables 4 and 5) and that of liver and kidney of Heteroclarias-fed rat (Tables 7 and 8). Jahanbakhshi and Hedayati (2012) reported that the components of oil can interact with the biosynthesis of pyridoxal phosphate, an essential molecule required for the normal functioning of aminotransferases. In this study, excessive utilization of energy by these animals to meet the increasing demand for survival under WSFD-induced stress might have altered the synthesis of this molecule and caused a disorder in the activities of the aminotransferases in their respective tissues. The reduction in blood glucose may also affect the function of the muscular tissues. Reduced level of glucose or hypoglycaemia have also been observed in other studies after exposure to crude oil and other chemical pollutants (Oluah *et al*., 2014, Isehunwa, *et al*., 2016; Owolabi and Omotosho, 2017). However, the observation of the present study contrasts the findings of Simonato *et al* (2008, 2013) who reported hyperglycaemic condition in *Prochilodus lineatus* following exposure to diesel oil and its derivative. Hence, exposure to the same toxicant in same or different organism can elicit different changes depending on the tolerance ability of the species to oxidative stress.

The decline in protein level in the experimental organisms could be as a result of breakdown of protein via the utilization of amino acids. In this study, the destruction of hepatocytes or necrosis and/or excessive loss of protein due to kidney damage with subsequent impairment of protein metabolic pathway might have also accounted for the depletion of protein levels in the tissues of experimental animals. The depletion of protein as glucose decreased may be due to the rapid utilization of protein in these tissues under oxidative stress condition (Owolabi and Omotosho, 2017). The probable impairment of glucose metabolism in the liver might have triggered the mobilization of body protein to provide additional energy as compensation through the process of deamination to form keto acids that are converted to pyruvate, which is utilized in the tricarboxylic acid cycle for energy production. Furthermore, the elevated patterns of ALT and AST in the sera of both animals which paralleled the depletion of protein could also be a compensatory mechanism to generate energy for detoxification process, tissue repairs and homeostatic maintenance. Transaminases have been reported to be concerned with energy metabolism which allow interplay among glucose, fat and protein metabolism to serve the changing demand of organism (Bell, 1972). Alterations in protein content of animals exposed to diesel oil and other pollutants have been reported in other studies (Khan *et al*., 2003, Yousafzai and Shakoori, 2011; Edori *et al*., 2013).

Enhanced LDH activity in the tissues of experimental animals in this study are similar to the findings of Oluah *et al* (2005) on *Clarias albopunctactus* exposed to increasing concentrations of Gammalin 20 and Actellic and Owolabi and Omotosho (2017) on *C. gariepinus*

exposed to atrazine. The significant increase in LDH activity in all the tissues investigated in both animals except serum is an indication that pyruvate, the end product of glycolysis, was channelled towards the lactic acid cycle to produce lactate and then to glucose to solve the energy crisis due to hypoxic stress. The decline in aminotransferase enzymes' activities and protein level and concurrent elevation in LDH activity in these tissues demonstrated that the animals under investigation were unable to tolerate the WSFD-induced oxidative stress and thus resulted to anaerobic means to survive the stress. This suggests that the respiratory activities of these animals was impaired and a coping strategy was developed by undertaking anaerobic oxidation to release energy via enhanced LDH activity.

Superoxide dismutase (SOD) constitutes the first line of defense against free radicals by catalyzing dismutation of superoxide anion radicals to water and hydrogen peroxide. In the present study, Heteroclarias exposure to WSFD polluted water and rat exposure through WSFD contaminated Heteroclarias diet suppressed the activity of SOD. The excessive production of superoxide radicals by the metallic and hydrocarbon constituents of diesel oil might have overwhelmed the antioxidant system of the experimental animals. This is in agreement with the works of Zhang, *et al*. (2004) on *Carassius auratus* and Farombi *et al* (2010) and Ogara *et al* (2016) on rat exposed to diesel oil, respectively.

Reduction in specific growth rate of experimental animals compared to their respective control could be due to loss of appetite or inefficient utilization of assimilated food. This might have resulted in lower retention of food nutrients in the body of the animals, hence the reduction in growth rate as the WSFD increased (Fig.1). These findings agree with Kori-Siakpere (2000) who noted that exposure of fish to water soluble fractions (WSF) of crude oil can result in reduced feeding and lower body weight. Ofojekwu and Onah (2002) reported that fish increase their metabolic rates to metabolize and excrete aromatic hydrocarbons and consequently allocate more energy to homeostatic maintenance than storage, thus exhibiting growth retardation. Similar observation was also made by Ogara *et al* (2016) in rat exposed to crude oil and Ikanone *et al* (2017) in rat fed crude oil contaminated diet. Donkins and Widdows (1986) reported that the body weight of organisms exposed to toxicants declined with respect to concentration of the toxicant and duration of exposure. Taylor *et al* (2000) also observed that organisms inhabiting oiled shore exhibited reduced body weight and elevated levels of biomarker than those in non-oiled areas.

CONCLUSION

The present study indicates that WSFD induced stress in both animals caused haematological, enzymatic and metabolic impairments. Based on this, the potential toxic effects of crude oil and its derivatives on aquatic organisms such as fish should be thoroughly monitored, so as to enhance the safety, survival and sustainable exploitation of healthy fish and also prevent the risk of health disorder in fish consumers.

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