Endocrine Disrupting Potential Of The Sub-Lethal Concentrations Of Primextra Herbicide On The Hormonal And Heamatological Profile Of *Clarias Gariepinus* (Burchell, 1822) Sub-Adults

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Abstract

The application of primextra on farmlands to curb weed growth contaminates water bodies, thereby resulting in endocrine disruptions in aquatic organisms. The sub-adults (n = 200) of *Clarias gariepinus* were exposed to 0.00 (control), 0.07, 0.14, 0.21, and 0.28 mg/L of primextra, and the hormonal and haematological alterations were observed over a period of 28 days. The alterations in the hormonal and haematological profile of the exposed fish was concentration dependent. The thyroxine (T4), tri-iodothyronine (T3), and estradiol (hormonal parameters) reduced significantly ($p<0.05$) compared to the control with increase in the concentration of the toxicant after four weeks of exposure. The T4, T3, and estradiol concentration decreased with increase in the exposure duration for the 0.07, 0.14, 0.21 and 0.28 mg/L group. The haemoglobin (Hgb), pack cell volume (PCV), red blood cells (RBC), white blood cells (WBC), mean corpuscular volume (MCV), and mean corpuscular heamoglobin concentration (MCHC) decreased significantly ($p<0.05$), while the mean corpuscular haemoglobin (MCH) increased significantly ($p<0.05$) from the control with increase in the concentration of the toxicant after four weeks of exposure. The Hgb, PCV, RBC, WBC, and MCV decreased, while MCH and MCHC in the fish increased with increase in the exposure duration for the 0.07, 0.14, 0.21 and 0.28 mg/L group. With the observed alterations in the hormonal and haematological parameters in the fish exposed to primextra, indiscriminate use of chemicals on farms is highly discouraged, in order to maintain a healthy terrestrial and aquatic eco-system, including our fishery resources and man.

Keywords: Endocrine disruption, sub-lethal, primextra, hormonal profile, heamatological parameters, *Clarias gariepinus*

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1. INTRODUCTION

The world's oceans will continue to maintain its integrity of providing ecosystem services to mankind, if environmental pollution will not cut short of its life expectancy of supporting aquatic species (Odoemelam *et al*., 2014). Environmental changes are often associated with human activities such as deforestation, release of industrial and domestic effluents, and even the use of pesticides in agricultural fields, which is one of sources that most contributes to the degradation of the quality of water resources (Odoemelam *et al*., 2014).

As one of the most globally distributed fisheries resources in aquatic ecosystems, fish is highly vulnerable to environmental contaminants and may give a reflection on the extent biological effects of these contaminants in water (Ramesh *et al*., 2009). The health of aquatic ecosystems have been globally evaluated using the biochemical changes in fishes, which is a reliable biomarker of environmental pollution (Schlenk and Di-Giulio, 2002). In tropical regions, *Clarias gariepinus* is the most commonly used species for aquaculture. The species is broadly spread and accepted by African farmers, because it grows to a very large size within a short period of time, tolerate unsuitable water quality, has low bone content, high yield, omnivorous feeding habit, and cope with overcrowding (Gunder, 2004). Their fry and fingerlings are readily available because they can be successfully propagated artificially, and this makes its market value high (Osman *et al*., 2006). It is a typical catfish species with air-breathing features, and survive for many hours out of the water and for many weeks in muddy marshes as well as capable of living even in most of these habitats subjected to seasonal dryness (Gunder, 2004).

Atrazine and S-metolachlor are the active ingredients of Primextra herbicide. It is a pre-emergence, broad spectrum herbicide and used extensively for the control of grasses and weeds in farms. This herbicide is commonly used by literate and illiterate farmers in Nigeria, since it is readily available in local markets. It has the capacity to cause groundwater and surface water contamination. Depending on the soil type, its half-life in the soil is usually within the range of 15–60 days (Extoxnet, 2007). The atrazine component of primextra formulation with name (2-Chloro-4-ethylamino-6-Isopropylamino-s-triazine) belongs to the s-triazine family, and is globally used for various weed control programmes (Ventura *et al*., 2008). It is commonly used for sorghum, sugar cane, corn, pineapples, especially in the rural areas. The metolachlor component of primextra, with name [2-chloro-N-(ethyl-6-methyl (phenyl)-N-(2-methoxy-1-methylethyl acetamide) is a chloroacetanilide herbicide, that is widely used as inhibitors of the growth of target weeds, by halting the preventing the production of fatty acids, chlorophyll, lipids, and proteins (Extoxnet, 2007).

The contamination of water bodies by pesticides either directly or indirectly kills fish, reduces their survival, growth rate, reproduction, and increases the concentration of these chemicals to undesirable levels in edible fish tissue (Rahman *et al*., 2002). As a result, humans who eat these contaminated fishes are exposed to several health issues (Rahman *et al*., 2002). Pesticides have endocrine disrupting potentials in biological organisms. Endocrine disrupting chemicals (EDCs) are chemical that alters the normal functioning of the endocrine system (McKinlay *et al*., 2008), with the natural chemicals like phytoestrogen or synthetic chemicals such as plasticizers, polychlorinated biphenyls (PCBs), Pesticides, and alkylphenolic compounds inclusive. EDCs causes abnormality either through mimicking and act like a natural hormone (Sonnenschein and Soto, 1998).

The evaluation of diverse biomarkers of environmental contamination in fishes, has been successfully used globally to raise early alarm on impending chemical exposure (Osman *et al*., 2010). Studies on the physiological states of species /animals using haematological analysis have been routinely revealed to determine the changes in environmental factors (Solomon and

Okomoda, 2012). Blood parameters are utilized as structural and functional component of a fish, to reveal exposure to contaminants, making it a patho-physiological indicators of the whole body (Maheswaran *et al*., 2008).

The knowledge on the sub-lethal effects of toxic chemicals on the hormonal and haemato-logical parameters is highly pertinent for outlining the health status of fish, to create an understanding on the future ecological impact. Primextra is a herbicide used extensively of recent in agricultural farmlands for weeds control in Nigeria and other African countries. However, no study exist on the sub-lethal effects of primextra on the hormonal and haematological alterations of *C. gariepinus*. To this end, this study is pertinent and will reveal the possible toxic effects of primextra on *C. gariepinus* sub-adults to the public, government, and the farmers, to ensure proper and adequate control of these chemicals in agricultural farmlands. This study will equally provide a reference line data through which studies in the future can be compared. The study evaluated the sub-lethal effects of primextra on some hormonal profiles triodothyronine (T3), 17 β estradiol (E2), and (thyroxine (T4) hormone) and haematological parameters mean white blood count (WBC), corpuscular haemoglobin concentration (MCHC), (packed cell volume (PCV), haemoglobin (HB), red blood cell (RBC), mean corpuscular haemoglobin (MCH), and mean corpuscular volume (MCV) of *C. gariepinus* sub-adults.

2. MATERIALS AND METHODS

2.1 Collection and transportation of fish/chemicals

C. gariepinus (n = 400) of sub-adults weight (180-200g) and length (36-40cm) were sourced from University of Calabar Fish farm, Cross River State, Nigeria. The fish samples collected were of the same genetic background and apparently healthy. The fish samples were collected in the morning between 7.30 and 9.00am and transported in a plastic container containing hatchery water to the Department of Zoology and Environmental Biology, Postgraduate Research Laboratory Unit, University of Calabar. The primextra (99% purity) was purchased from Ministry of Agriculture, Calabar, Cross River State, Nigeria.

2.2 Acclimation and maintenance of fish

In the laboratory, samples were acclimated to laboratory conditions over a period of 14 days in four transparent rectangular plastic aquaria measuring 60 x 29 x 28 cm filled in 25 liters of dechlorinated water. During the acclimation, the culture waters were changed daily and during this phase of the study, and daily monitoring of fish mortality was equally carried-out (Adetola, 2011). The experimental fish were fed daily with 2mm of commercial fish feeds (coppens) at 4% of their body weight. The unconsumed feeds were removed using scoop net to avoid test medium contamination. Before commencement of the experiment, feeding was discontinued for 24hours period.

2.3 Preparation of stock solutions

A standard method in bioassay was employed (OECD, 2002). The stock solution was prepared by mixing 0.15 mL of primextra with 999.85 mL of distilled water in a one liter container (Reish and Oshida, 1987). From the stock solution, serial dilutions of the toxicants were prepared.

2.4 Experimental design

After the acclimation period, the fish samples were divided into five groups of plastic aquaria and 20 fishes were introduced into each aquarium containing 25L of dechlorinated water (two replicates per aquaria/treatment). The sub-adults were divided into two groups. The first group of 200 sub-adults were used for the LC_{50} assay and the other 200 were allocated to the four treatment groups of the sub-lethal experiment. Each of the four aquaria of the treatment groups (LC_{50} assay and sub-lethal treatments) and control in duplicate contained 20 fish per each aquarium. The 96hr LC_{50} of varied the concentrations of primextra was carried out under controlled laboratory conditions.

The sub-adults of the treatment groups were exposed to sub-lethal concentrations of the toxicant in duplicate. To each aquarium of the treatment group, 5%, 10%, 15%, 20% of 96 hr

LC_{50} of the stock solution of primextra was added to 25 liters of the culture water.

2.5 Sub-lethal exposure

The exposure of *C. gariepinus* sub-adults to sub-lethal concentrations of primextra were conducted following the guidelines stipulated for fish toxicity testing by (OECD, 2002). The fish samples were exposed to 0.07, 0.14, 0.21, and 0.28 mg/L of primextra (which were 5%, 10%, 15%, and 20% of the 96h-LC_{50} value of 1.4 mg/l for the test chemical) for 28 days. The control was not exposed the toxicant. The exposure was done in two replicates per test concentration, to ensure an accurate result. Each of the test aquaria contained equal numbers of fish (20) and replicate of the test aquaria were separated physically. During the experiment proper, the fishes were fed with 2mm of coppens at 4% of their body weight twice daily and the test medium renewed every 48hours with a new solution of these toxicants added to restore the desired concentration on the plastic aquaria.

2.6 Sample collection

Fish samples were collected randomly from each of the aquaria exposed to varied concentrations after 7, 14, 21, and 28 days exposure period. Fish samples were anaesthetized on immersing on chill water bathe. About 2 mL of blood samples were collected using a 5 mL heparinsed syringe as recommended by Blaxhall and Daisley (1973) to prevent blood coagulation for haematological assay. Another 4 mL of blood samples were collected with 5 mL syringe, which were transferred into a syringe containing lithium heparin for hormonal assays.

2.7 Hormonal assay

The collected blood samples were transferred into plain tube and allow for coagulation. Serum was obtained by spinning the blood sample at 3,000 rpm for 10mins. The supernatant serum were transferred into a dry labeled and stoppered micro-centrifuge tube and stored at 2°C to 8°C for hormonal profile estimation. Mini-Vidas T3, T4 and estradiol test kits using automated mini-vidas analyzer (Bio-merieux Instruments, Inc. Germany) were employed for the determination of the activity of serum T3, T4 and estradiol levels after every 7 days interval for 28 days of the experiment. Hormonal parameters were analysed at the University of Calabar teaching hospital, Cross River State, Nigeria.

2.8 Haematological assay

The collected blood samples were transferred into sample bottles containing EDTA solution for the analysis of haematological parameters such as the RBC, HB, PCV, WBC, MCV, MCH, and MCHC using Haemolyzer-Sysmex model: XS-1000i after every 7days interval for 28days (4weeks) of the experiment. Haematological indices according to Dacie and Lewis (2001) was also calculated. Haematological parameters were analysed at the haematological unit of the University of Calabar teaching hospital, Cross River State, Nigeria.

2.9 Statistical analysis

The data obtained from the haematological and hormonal parameters were subjected to descriptive statistics (mean and standard deviation). Analysis of variance (ANOVA) was used to test for the significance of differences in the changes in hormonal and haematological parameters between each group exposed to different concentrations of primextra after 7, 14, 21, 28 days of exposure period compared to control at 0.05 levels of significance. All analyses were performed using predictive analytical software (PASW 25.0).

3. RESULTS

The changes in hormonal and haematological parameters of the sub-adults of *Clarias gariepinus* exposed to sub-lethal concentrations of primextra is presented in Tables 1- 10 and Figures 1- 2. The summary of the hormonal levels of *C. gariepinus* exposed to different concentrations of primextra for four weeks is shown in Tables 1-3 and variations in mean value of hormonal level of primextra is shown in Figure 1.

3.1 Hormonal profile of *C. gariepinus* exposed to primextra

3.1.1. Changes in thyroxine (T4) levels

Primextra induced a significant change in the T4 of *C. gariepinus* after four weeks (28 days) of exposure (Table1). The T4 values reduced significantly from the control with increase in the concentration of the toxicant after four weeks of exposure ($p<0.05$) (Figure 1).

After exposure of *C. gariepinus* to different sub-lethal concentration of primextra, the T4 values decreased from 34.10±0.20 (control) to 29.37±0.75 (0.28 mg/L) after one week, 30.77±2.26 (control) to 22.50±2.96 (0.28 mg/L) after two weeks, 27.63±1.56 (control) to 17.60±0.44 (0.28 mg/L) after three weeks, and from 33.20±0.72 (control) to 14.80±0.60 (0.28 mg/L) after four weeks of exposure (Table1). The T4 decreased significantly with increase in the exposure period for the 0.07, 0.14, 0.21 and 0.28 mg/L group at $p<0.05$ (Table1).

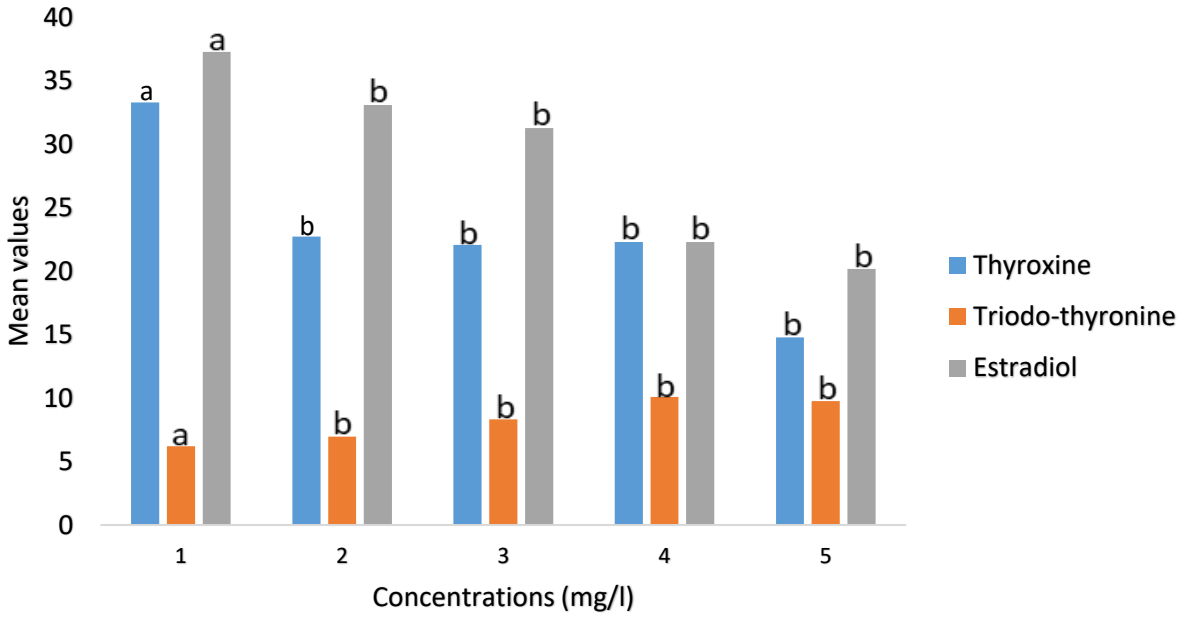

Fig. 1: Variation in the hormonal levels of *C. gariepinus* exposed to primextra for four weeks
Note: The different superscript of all hormones for each concentration (concentration 1: control, concentration 2: 0.07 mg/L, concentration 3: 0.14 mg/L, concentration 4: 0.21 mg/L, and concentration 5: 0.28 mg/L) compared to the control are significantly different ($p<0.05$)

Table 1: Alterations in thyroxine (T4) of *C. gariepinus* exposed to different concentration of primextra for four weeks (28 days)

Hormone	Duration (weeks)	Control 0.0	0.07mg/L	0.14 mg/L	0.21 mg/L	0.28 mg/L
Thyroxine $T_{4\ nm0}{}^{1}/_{L}$	Week 1	34.10±0.20^{a}	31.60±1.44^{a}	29.57±0.55^{a}	29.47±0.50^{a}	29.37±0.75^{a}
	Week 2	30.77±2.26^{b}	27.50±1.74^{b}	25.23±0.96^{b}	22.67±0.72^{b}	22.50±2.96^{b}
	Week 3	27.63±1.56^{c}	24.60±1.71^{c}	25.50±0.99^{c}	23.17±0.78^{c}	17.60±0.44^{c}
	Week 4	33.20±0.72^{d}	22.73±1.36^{d}	22.07±1.92^{d}	22.30±0.85^{d}	14.80±0.60^{d}

Values with different superscript within the same concentration for each week are significantly different at $p<0.05$

3.1.2 Changes in tri-iodothyronine (T3) levels

Primextra induced a significant change in the T3 of *C. gariepinus* after four weeks (28 days) of exposure (Table 2). T3 reduced significantly from the control with increase in the concentration of the toxicant after four weeks of exposure ($p<0.05$) (Figure 1).

After exposure of *C. gariepinus* to different sub-lethal concentration of primextra, the T3 values increased from 1.40±0.30 (control) to 3.43±0.12 (0.28 m*g*/L) after one week, 2.67±0.23 (control) to 6.43±0.15 (0.28 m*g*/L) after two weeks, 4.47±0.31 (control) to 9.23±0.15 (0.28 m*g*/L) after three weeks, and from 6.20± 0.20 (control) to 9.77±0.25 (0.28 mg/L) after four weeks of exposure (Table 2). The T3 values increased insignificantly with increase in the exposure period for the 0.14 mg/L to 0.28 m*g*/L group ($p<0.05$), but decreased insignificantly with increase in the exposure period for the 0.00 and 0.07 m*g*/L group at $p>0.05$ (Table 2).

Table 2: Changes in tri-iodothyronine (T3) of *Clarias gariepinus* exposed to different concentration of Primextra for four weeks (28 days)

Hormone	Duration (weeks)	Control 0.0	0.07mg/L	0.14 mg/L	0.21 mg/L	0.28 mg/L
Triodo-thyronine T3	Week 1	1.40±0.30^{a}	2.47 ±0.15^{b}	2.67±0.15^{a}	3.13±0.15^{a}	3.43±0.12^{a}
	Week 2	2.67±0.23^{a}	4.17±0.21^{b}	5.03±0.15^{b}	5.03±0.12^{b}	6.43±0.15^{b}
	Week 3	4.47±0.31^{a}	6.00±0.20^{b}	6.97±0.15^{c}	7.20±0.10^{c}	9.23±0.15^{c}
	Week 4	6.20± 0.20^{a}	6.97±0.15^{b}	8.33±0.12^{d}	10.1± 0.23^{d}	9.77±0.25^{d}

Values with different superscript within the same concentration for each week are significantly different at $p<0.05$ and values with the same superscript within the same concentration for each week are not significantly different at $p>0.05$

4.1.3 Changes in estradiol levels

Primextra induced a significant change in the estradiol values of *C. gariepinus* after four weeks (28 days) of exposure (Table 3). Estradiol values reduced significantly from the control with increase in the concentration of the toxicant after four weeks of exposure ($p<0.05$) (Figure 1).

After exposure of *C. gariepinus* to different sub-lethal concentrations of primextra, the estradiol values decreased from 34.18±0.35 (control) to 24.0±0.06 (0.28 m*g*/L) after one week, 42.4±0.95 (control) to 23.5±0.42 (0.28 m*g*/L) after two weeks, 37.7±0.46 (control) to 21.5±0.23 (0.28 m*g*/L) after three weeks, and from 37.3± 0.06 (control) to 20.2±0.15 (0.28 m*g*/L) after four weeks of exposure (Table 3). The estradiol decreased significantly with increase in the exposure period for the control group ($p<0.05$), and also significantly decrease in the exposure period for the 0.07, 0.14, 0.21 and 0.28 m*g*/L group at $p<0.05$ (Table 3).

Table 3: Alterations in estradiol of *Clarias gariepinus* exposed to different concentration of primextra for four weeks (28 days)

Hormone	Duration (weeks)	Control 0.0	0.07mg/L	0.14 mg/L	0.21 mg/L	0.28 mg/L
Estradiol $_{E\ Pg^{1}/L}$	Week 1	41.8±0.35^{a}	39.3 ±0.12^{a}	36.2±0.35^{a}	34.1±0.17^{a}	24.0±0.06^{a}
	Week 2	42.4±0.95^{b}	37.2±0.17^{b}	36.2±0.20^{b}	30.4±0.72^{b}	23.5±0.42^{b}
	Week 3	37.7±0.46^{c}	35.3±0.12^{c}	33.1±0.12^{c}	29.8±0.35^{c}	21.5±0.23^{c}
	Week 4	37.3±0.06^{d}	33.1±0.10^{d}	31.3±0.12^{d}	22.3±0.17^{d}	20.2±0.15^{d}

Values with different superscript within the same concentration for each week are significantly different at $p<0.05$

3.2 Haematological levels of *C. gariepinus* exposed primextra

The summary of the haematological levels of *C. gariepinus* exposed to different concentrations of primextra for four weeks is shown in Table 4 -10 and the variations in mean value of haematological level of primextra is shown in Figure 2.

3.2.1 Changes in haemoglobin (Hgb) content levels

Primextra induce a significant change in the Haemoglobin content of *C. gariepinus* after four weeks (28 days) of exposure (Table 4). Haemoglobin values reduced significantly from the control with increase in the concentration of the toxicant after four weeks of exposure ($p<0.05$) (Figure 2).

After exposure of *C. gariepinus* to primextra, the haemoglobin values decreased from 8.23±0.25 (control) to 6.33±0.12 (0.28 m*g*/L) after one week, 8.27±0.23 (control) to 4.30±0.10 (0.28 m*g*/L) after two weeks, 9.27±0.55 (control) to 4.23±0.21 (0.28 m*g*/L) after three weeks, and from 9.37± 0.06 (control) to 3.47±0.12 (0.28 m*g*/L) after four weeks of exposure (Table 4). The haemoglobin values decreased significantly with increase in the exposure period for the 0.00, 0.07, 0.14, 0.21, 0.28 m*g*/L group for week 3 and 4 ($p<0.05$), but decreased insignificantly with increase in the exposure period for the 0.00, 0.07 and 0.14mg/l group for week 1 and 2 at $p>0.05$ (Table 4).

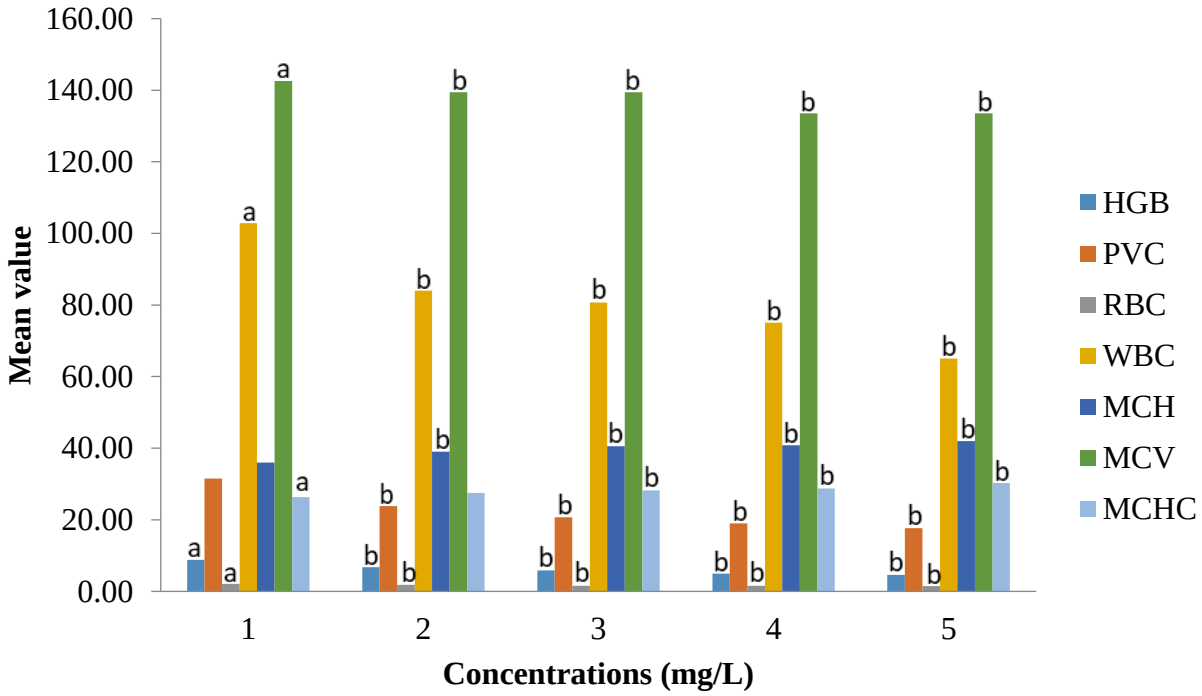

Fig. 2: Variation in mean value of haematological levels in primextra exposed to sub-lethal concentration (mg/L) of *C. gariepinus* for four weeks

Note: The different superscript of all haematological parameters for each concentration (concentration 1: control, concentration 2: 0.07 mg/L, concentration 3: 0.14 mg/L, concentration 4: 0.21 mg/L, and concentration 5: 0.28 mg/L) compared to the control are significantly different ($p<0.05$)

Table 4: Changes in haemoglobin (Hgb) of *C. gariepinus* exposed to different concentrations of primextra for 4 weeks (28 days)

Blood parameters	Duration (weeks)	Control 0.0	0.07mg/L	0.14 mg/L	0.21 mg/L	0.28 mg/L
HGB (g/dL)	Week 1	8.23±0.25^{a}	7.07±0.12^{a}	7.33±0.12^{c}	6.73±0.24^{d}	6.33±0.12^{e}
	Week 2	8.27±0.23^{b}	7.07±0.06^{b}	6.53±0.12^{c}	5.27±0.23^{d}	4.30±0.10^{e}
	Week 3	9.27±0.55^{c}	6.80±0.20^{c}	5.13±0.23^{c}	4.27±0.12^{d}	4.23±0.21^{e}
	Week 4	9.37±0.06^{d}	6.10±0.17^{d}	4.33±0.29^{c}	3.53±0.23^{d}	3.47±0.12^{e}

Values with different superscript within the same concentration for each week are significantly different at $p<0.05$ and values with the same superscript within the same concentration for each week are not significantly different at $p>0.05$

3.2.2 Changes in pack cell volume (PCV) levels

Primextra induced a significant change in the PCV of *C. gariepinus* after four weeks (28 days) of exposure (Table 5). The PCV values reduced significantly from the control with increase in the concentration of the toxicant after four weeks of exposure ($p<0.05$) (Figure 2).

After exposure of *C. gariepinus* to different sub-lethal concentration of primextra, the PCV values decreased from 31.5±0.26 (control) to 23.2±0.17 (0.28 m*g*/L) after one week, 32.77±0.40 (control) to 18.2±0.06 (0.28 m*g*/L) after two weeks, 32.90±0.62 (control) to 16.3±0.23 (0.28 m*g*/L) after three weeks, and from 28.90± 0.46 (control) to 13.1±0.12 (0.28 m*g*/L) after four weeks of exposure (Table 5). The PCV values decreased significantly with increase in the exposure period for the control group ($p<0.05$), and also significantly decrease in the exposure period for the 0.07, 0.14, 0.21 and 0.28 m*g*/L group at $p<0.05$ (Table5).

Table 5: Changes in PCV of *C. gariepinus* exposed to different concentration of primextra for 4 weeks (28 days)

Blood parameter	Duration (weeks)	Control 0.0	0.07mg/L	0.14 mg/L	0.21 mg/L	0.28 mg/L
PCV	Week 1	31.5±0.26^{a}	27.53 ±0.23^{a}	24.33±0.15^{a}	24.1±0.12^{a}	23.2±0.17^{a}
	Week 2	32.77±0.40^{b}	24.67±0.46^{b}	21.40±0.87^{b}	20.73±0.31^{b}	18.2±0.06^{b}
	Week 3	32.90±0.62^{c}	22.80±0.35^{c}	19.77±0.51^{c}	17.10±0.17^{c}	16.3±0.23^{c}
	Week 4	28.90±0.46^{d}	20.47±0.46^{d}	17.40±0.00^{d}	14.27±0.12^{d}	13.1±0.12^{d}

Values with different superscript within the same concentration for each week are significantly different at $p<0.05$

3.2.3 Changes in red blood cells (RBC) levels

Primextra induced a significant change in the RBC of *C. gariepinus* after four weeks (28 days) of exposure (Table 6). RBC values reduced significantly from the control with increase in the concentration of the chemical after four weeks of exposure ($p<0.05$) (Figure 2).

After exposure of *C. gariepinus* to different sub-lethal concentration of primextra, the RBC values decreased from 2.20±0.01 (control) to 1.70±0.006 (0.28 m*g*/L) after one week, 2.00±0.01 (control) to 1.42±0.015 (0.28 mg/L) after two weeks, 2.13±0.01 (control) to 1.30±0.006 (0.28 m*g*/L) after three weeks, and from 2.13± 0.01 (control) to 1.41±0.012 (0.28 m*g*/L) after four weeks of exposure (Table 6). The RBC values decreased significantly with

increase in the exposure period for the 0.14, 0.21 and 0.28 m*g*/l group ($p<0.05$), but decreased insignificantly with increase in the exposure period for the 0.00 and 0.07 mg/l group at $p>0.05$ (Table 6).

Table 6: Changes in RBC of *C. gariepinus* exposed to different concentration of primextra for 4 weeks (28 days)

Blood parameter	Duration (weeks)	Control 0.0	0.07m*g*/L	0.14 m*g*/L	0.21 m*g*/L	0.28 m*g*/L
RBC	Week 1	2.20±0.01^{a}	1.97±0.02^{b}	1.91±0.01^{c}	1.80±0.01^{d}	1.70±0.006^{a}
	Week 2	2.00±0.01^{a}	1.84±0.01^{b}	1.61±0.01^{c}	1.61±0.01^{d}	1.42±0.015^{b}
	Week 3	2.13±0.01^{a}	1.77±0.01^{b}	1.52±0.02^{c}	1.51±0.01^{d}	1.30±0.006^{c}
	Week 4	2.13±0.01^{a}	1.81±0.02^{b}	1.33±0.02^{c}	1.32±0.01^{d}	1.41±0.012^{d}

Values with the same superscript within the same concentration for each week are not significantly different at $p>0.05$ and Values with different superscript within the same concentration for each week are significantly different at $p<0.05$

3.2.4 Changes in white blood cells (WBC) levels

Primextra induced a significant change in the WBC of *C. gariepinus* after four weeks (28 days) of exposure (Table 7). The WBC values reduced significantly from the control with increase in the concentration of the toxicant after four weeks of exposure ($p<0.05$) (Figure 2).

After exposure of *C. gariepinus* to different sub-lethal concentration of primextra, the WBC values decreased from 122.1±0.06 (control) to 108.70±1.38 (0.28 mg/L) after one week, 113.0±0.35 (control) to 74.20±0.00 (0.28 mg/L) after two weeks, 84.27±0.06 (control) to 43.97±0.15 (0.28 mg/L) after three weeks, and from 92.27±0.12 (control) to 33.23±0.06 (0.28 mg/L) after four weeks of exposure (Table 7). The WBC values decreased significantly with increase in the exposure period for the control group ($p<0.05$), and also significantly decrease in the exposure period for the 0.07, 0.14, 0.21 and 0.28 m*g*/L group at $p<0.05$ (Table7).

Table 7: Alterations in WBC of *C. gariepinus* exposed to different concentration of primextra for 4 weeks (28 days)

Blood parameter	Duration (weeks)	Control 0.0	0.07m*g*/L	0.14 m*g*/L	0.21 m*g*/L	0.28 m*g*/L
WBC	Week 1	122.1±0.06^{a}	120.3±0.12^{a}	115.13±0.29^{a}	94.07±0.12^{a}	108.70±1.38^{a}
	Week 2	113.0±0.35^{b}	100.27±0.12^{b}	101.37±0.06^{b}	94.07±0.12^{b}	74.20±0.00^{b}
	Week 3	84.27±0.06^{c}	61.33±0.06^{c}	60.10±0.17^{c}	54.13±0.12^{c}	43.97±0.15^{c}
	Week 4	92.27±0.12^{d}	54.07±0.12^{d}	46.07±0.12^{d}	41.13±0.12^{d}	33.23±0.06^{d}

Values with different superscript within the same concentration for each week are significantly different at $p<0.05$

3.2.5 Changes in the mean corpuscular haemoglobin (MCH) levels

Primextra induced a significant change in the MCH of *C. gariepinus* after four weeks (28 days) of exposure (Table 8). The MCH values increased significantly from the control with increase in the concentration of the toxicant after four weeks of exposure ($p<0.05$) (Figure 2).

After exposure of *C. gariepinus* to different sub-lethal concentration of primextra, the MCH values increased from 38.6±0.00 (control) to 39.0±0.06 (0.28 mg/L) after one week, increased from 36.2±0.06 (control) to

41.9±0.012 (0.28 mg/L) after two weeks, 34.6±0.25 (control) to 43.0±0.00 (0.28 mg/L) after three weeks, and from 34.6± 0.00 (control) to 43.9±0.27 (0.28 mg/L) after four weeks of exposure (Table 8). The MCH values decreased significantly with increase in the exposure period for the control group ($p<0.05$), but insignificantly increase in the exposure period for the 0.07, 0.14, 0.21 and 0.28 mg/L group at $p<0.05$ (Table 8)

Table 8: Changes in MCH of *C. gariepinus* exposed to different concentration of primextra for 4 weeks (28 days)

Blood parameter	Duration (weeks)	Control 0.0	0.07mg/L	0.14 mg/L	0.21 mg/L	0.28 mg/L
MCH	Week 1	38.6±0.00^{a}	38.6±0.23^{a}	38.7±0.06^{a}	38.7±0.12^{a}	39.0±0.06^{a}
	Week 2	36.2±0.06^{b}	39.2±0.06^{b}	40.3±0.12^{b}	41.5±0.06^{b}	41.9±0.12^{b}
	Week 3	34.6±0.25^{c}	39.5±0.12^{c}	41.1±0.12^{c}	40.9±0.06^{c}	43.0±0.00^{c}
	Week 4	34.6±0.00^{d}	39.1±0.12^{d}	42.3±0.06^{d}	42.2±0.00^{d}	43.9±0.27^{d}

Values with different superscript within the same concentration for each week are significantly different at $p<0.05$

3.2.6 Changes in Mean corpuscular volume (MCV) levels

Primextra induced a significant change in the mean cell volume of *C. gariepinus* after four weeks (28 days) of exposure (Table 9). The MCV values reduced significantly from the control with increase in the concentration of the chemical after four weeks of exposure ($p<0.05$) (Figure 2).

After exposure of *C. gariepinus* to different sub-lethal concentration of primextra, the MCV values decreased from 142.7±0.06 (control) to 142.1±0.06 (0.28 mg/L) after one week, 144.2±0.06 (control) to 136.1±0.12 (0.28 mg/L) after two weeks, 142.1±0.06 (control) to 134.0±0.00 (0.28 mg/L) after three weeks, and from 141.2± 0.00 (control) to 122.1±0.06 (0.28 mg/L) after four weeks of exposure (Table 9). The MCV values decreased insignificantly with increase in the exposure period for the control ($p<0.05$), and also insignificantly decrease in the exposure period for the 0.07, 0.14, 0.21mg/l, but decreased significantly for 0.28 mg/L group at $p<0.05$ (Table 9).

Table 9: Changes in MCV of *C. gariepinus* exposed to different concentration of primextra for 4 weeks (28 days)

Blood parameter	Duration (weeks)	Control 0.0	0.07mg/L	0.14 mg/L	0.21 mg/L	0.28 mg/L
MCV	Week 1	142.7±0.06^{a}	142.2±0.75^{a}	141.5±0.17^{a}	142.1±0.06^{a}	142.1±0.06^{a}
	Week 2	144.2±0.06^{b}	141.4±1.13^{b}	140.1±0.06^{b}	136.1±0.12^{b}	136.1±0.12^{b}
	Week 3	142.1±0.06^{c}	140.9±0.67^{c}	140.1±0.06^{c}	134.0±0.00^{c}	134.0±0.00^{c}
	Week 4	141.2±0.00^{d}	133.2±2.59^{d}	136.1±0.06^{d}	122.1±0.06^{d}	122.1±0.06^{d}

Values with different superscript within the same concentration for each week are significantly different at $p<0.05$

3.2.7 Changes in mean corpuscular haemoglobin concentration (MCHC) levels

Primextra induced a significant change in the MCHC of *C. gariepinus* after four weeks (28 days) of exposure (Table 10). The MCHC values reduced significantly from the control

with increase in the concentration of the chemical after four weeks of exposure ($p<0.05$) (Figure 2).

After exposure of *C. gariepinus* to different sub-lethal concentration of primextra, the MCHC values decreased from 27.3±0.06 (control) to 27.43±0.06 (0.28 mg/L) after one week, and increased from 26.07±0.06 (control) to 30.40±0.00 (0.28 mg/L) after two weeks, 26.17±0.06 (control) to 31.03±0.06 (0.28 mg/L) after three weeks, and from 26.07± 0.06 (control) to 32.07±0.06 (0.28 mg/L) after four weeks of exposure (Table 10). The MCHC values decreased insignificantly with increase in the exposure period for the control group ($p<0.05$), and also significantly increased in the exposure period for the 0.07, 0.14, 0.21 and 0.28 m*g*/L group at $p<0.05$ (Table 10).

Table 10: Changes in MCHC of *C. gariepinus* exposed to different concentration of primextra for 4 weeks (28 days)

Blood parameter	Duration (weeks)	Control 0.0	0.07mg/L	0.14 mg/L	0.21 mg/L	0.28 mg/L
MCHC	**Week 1**	27.13±0.06^{a}	27.1±0.10^{a}	27.1±0.06^{a}	27.2±0.06^{a}	27.43±0.06^{a}
	Week 2	26.07±0.06^{b}	27.3±0.06^{b}	27.7±0.06^{b}	27.2±0.06^{b}	30.40±0.00^{b}
	Week 3	26.17±0.06^{c}	27.7±0.23^{c}	28.1±0.06^{c}	29.4±0.00^{c}	31.03±0.06^{c}
	Week 4	26.07±0.06^{d}	28.2±0.06^{d}	29.8±0.06^{d}	31.1±0.12^{d}	32.07±0.06^{d}

Values with different superscript within the same concentration for each week are significantly different at $p<0.05$

4. DISCUSSION

The nature and health status of any aquatic population depends on the quality of the water bodies. In recent years, the increase in the concentration of toxic chemicals in the aquatic ecosystems has coincided with the rapid population growth globally and the continual technological advancement and industrialization to produce chemicals such as fertilizers and pesticides (Jesus and Carvalho, 2008). This study revealed for the first time, the endocrine disrupting potential of primextra on the hormonal and haematological profile of *C. gariepinus* sub-adult after 28 days of exposure, as biomarkers of toxicant induced health effect.

The health status of an aquatic environment and physiological changes in fishes are globally utilized to predict the contamination of the environment (Salazar-Lugo *et al.*, 2013). Fishes are consistently used as sentinel organisms for toxicity studies, due to the prominent role they play in trophic web, low concentration mutagen response, and accumulation of toxic substances (Cavas and Ergene-Gözükara, 2005). The exposure of the sub-adults of *C. gariepinus*, to sub-lethal concentrations of primextra, indicates its toxicity on the exposed fish with changes in various investigations carried-out. The observed changes after exposure to relatively low concentrations of primextra triggered a negative health status on sub-adults, thereby resulting in changes in hormonal and haematological alterations in the exposed fish. According to the present study, significant changes ($p<0.05$) in the hormonal profile levels was recorded in the fish with increase in the concentration of primextra, compared to the control group. The hormonal alterations in the sub-adults of *C. gariepinus* exposed to the sub-lethal concentration of primextra was concentration dependent, thereby corroborating with the findings of Joseph *et al*. (2019). This resulted in the decrease in the thyroxine (T4), estradiol, and a significant increase in triodothyronine (T3) concentrations compared to the control. This could be due to dysfunction of the thyroid connected to the 5' deiodonases inhibition, with reduced free T3 and increased reverse T3. The significant decrease in plasma T4 and a significant increase in T3 level of the fish during sub-lethal exposure to primextra, potentially resulted from the interruption of many sub-cellular components of the central nervous system. In the present study, the

estradiol levels in the fish decreased significantly with increase in the concentration of the toxicant compared to the control. The decrease in the estradiol concentration in the exposed *C. gariepinus* is in contrary to the findings of Ismail *et al*. (2016), and this could be due to the fact the hormonal level of estradiol differs depending on the reproductive cycle of fish (Joseph et al., 2019) and also due to the interfering with the free cholesterol production, testosterone-converted sex hormone precursor (Garcia-Reyero *et al*., 2006). This discrepancy between the compared studies could also be due to the difference in age of fish, frequency of exposure, toxicity of the chemical, duration of exposure, season, sex and species (Joseph *et al.*, 2019). On the contrary, the decrease in the hormonal concentrations of *C. gariepinus* with increase in the concentrations of primextra corroborated with the findings of Ghada, (2009) for the effect of butachlor on *O. niloticus*, Rodae-Ortiz *et al*. (2009) for a study on the alterations of estradiol concentration in male Nile tilapia, Ozcan-Oruc (2010) for a study on steroid hormone concentration in *O. niloticus* exposed to chlorpyrifos, Dogan and Can (2011) for a study on the endocrine disruption in *Oncorhynchus mykiss* exposed to dimethoate, and Joseph *et al*. (2019) for a study on sex hormones alterations in *C. gariepinus* exposed to cypermethrin. This confirms the potential of primextra to alter the hormonal profile of biological organisms.

Similar to the trend observed for the hormonal profile of the fish exposed to the studied toxicant, the changes in the heamatological profile was concentration dependent, resulting in the significant decrease ($p<0.05$) in HGB, PCV, RBC, and MCV concentrations. Similar observations were made by Jayaprakash and Shettu (2013), while studying the alterations in some hematological components of *Channa punctatus* exposed to different concentrations of deltametrin and Nwani *et al*. (2015) while studying physiological effect of paraquat in juvenile *C. gariepinus*. The significant decrease could be as a result of the responses that reduces the oxygen carrying capacity for the purpose of maintaining gas exchange in the damage gill lamellae and the generation of reactive oxidation stress due to the toxicant, which impose severe oxidative stress on the fish (Mazouk *et al*., 2012). This agreed with the findings of Ramesh *et al*. (2009) and Agbon (2014), which showed significantly decreased concentrations of RBC, PCV, HB compared with control group. To this end, the significant decrease in the RBC counts could induce anaemia, attributable to erythrocytes destruction or hemosynthesis and erythropoiesis inhibition, an indication that the fishes were stressed during the sub-lethal exposure to the primextra concentrations. The significant decrease in the WBC and HGB counts agreed with finding reported by Mazouk *et al*. (2012) for a study on the effect of atrazine to female catfish. However, the relative decrease in the PCV and MCV observed in this study disagreed with findings reported by Korisiakpere *et al*. (2007) for a study on the haematological alterations in *C. gariepinus* exposed to paraquat. The discrepancy is attributable to the difference in the concentration of the used pesticides, fish species, age, and size (Joseph *et al*., 2019). According to Agbon (2014), the sub- lethal exposure of fish revealed significant decrease in the PCV which induced anaemia and macrocytosis. This leads to the destruction of erythrocytes or the inhibition of erythropoiesis and hemosynthesis as shown in the significant decrease in RBC count, thereby indicating that the fishes were stressed in the course of the exposure to primextra. The decrease in the PCV values may also be due to response to stress imposed on them, an indication that primextra treatment potentially interfered with the normal RBC and HB physiology. The significant decrease in HB levels observed in the present study could equally be attributed to either an increased rate through which the HGB is destroyed or a decline in the HGB rate synthesis under anoxic conditions, which allows depression and exhaustion in haemopoietic potentials to occur (Marzouk *et al*., 2012). The MCV values decrease significantly ($p<0.05$) across the different treatments groups compared with control, which is an indication of macrocytosis and hypocromic anaemia. The present study equally recorded significant decrease ($p<0.05$)

in the WBC of the exposed fish compared to the control. This could be attributed to sudden changes predominantly arising in the polymorph nuclear WBC, a phenomenon that coincided with oesinopenia, monocytosis, and a viable degree of lymphopenia. The WBC decrease may also be as a result of the swelling of erythrocyte or haemolysis from the increase in the protein-carbondioxide in the blood of the fish. This agreed with the findings of Marzouk *et al*. (2012), where the decrease in WBC was attributed to anaemia possibly due to the haemodilution emanating from impaired osmoregulation across the gill epithelium. Furthermore, the decrease in the erythropoietic activity of the haemodilution or kidney across the epithelium and decrease of the non-specific immunity in fish after exposure to pesticide may also be responsible in the decrease in the WBC level of *C. gariepinus* exposed to primextra (Svoboda *et al*., 2007). The significant decrease in the in the WBC compared to the control observed in the present study is contrary to the findings of Nwani *et al.* (2015) who reported a significant increase in white blood cell. This differences in the profile of WBC between the two studies is attributable to the difference in fish species, age, the cycle of sexual maturity and health condition of the fish (Vaiyanan *et al*., 2015).

5. CONCLUSION

Findings in this study revealed that the exposure of fish to sub-lethal concentrations of primextra results in possible hormonal dysfunction and changes in haematological profiles leading to anemia. The alterations in the hormonal and haematological parameters of the fish exposed to primextra was concentration dependent. Even at low concentration, primextra in the environment is still injurious to the aquatic ecosystem and man. Although, these concentrations may not be fatal but can lead to salient changes in the physiology of the fish and eventually affect their survival. This study also revealed that primextra is toxic even at low concentrations when ingested or absorbed by fish over a period of time.

COMPETING INTERESTS

Authors have declared that there is no competing interest or whatsoever to declare.

ETHICAL APPROVAL

Prior to the commencement of the study, approval was gotten for animal usage in experiments in accordance with the 1986 Acts and associated guidelines of U.K. for the use of Animals in scientific experiments, EU Directive 2021/83/EU for animal experiment-tations.

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AUTHORS CONTRIBUTION

Akaninyene Joseph and Andem Bassey took up the Conceptualization; Ebari Sylvanus handled Data curation; Raymond Ajang and did the Formal analysis; George Eni handled Funding acquisition; Akaninyene Joseph and Andem Bassey handled Investigation; Akaninyene Joseph drafted the Methodology; Andem Bassey carried out Project administration; Ebari Sylvanus took care of Resources; Akaninyene Joseph handled Software; George Eni handled Supervision; Raymond Ajang took care of Validation; Ebari Sylvanus carried out Visualization; Akaninyene Joseph handled Roles/Writing - original draft; Akaninyene Joseph and Andem Bassey did the Writing - review & editing.

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