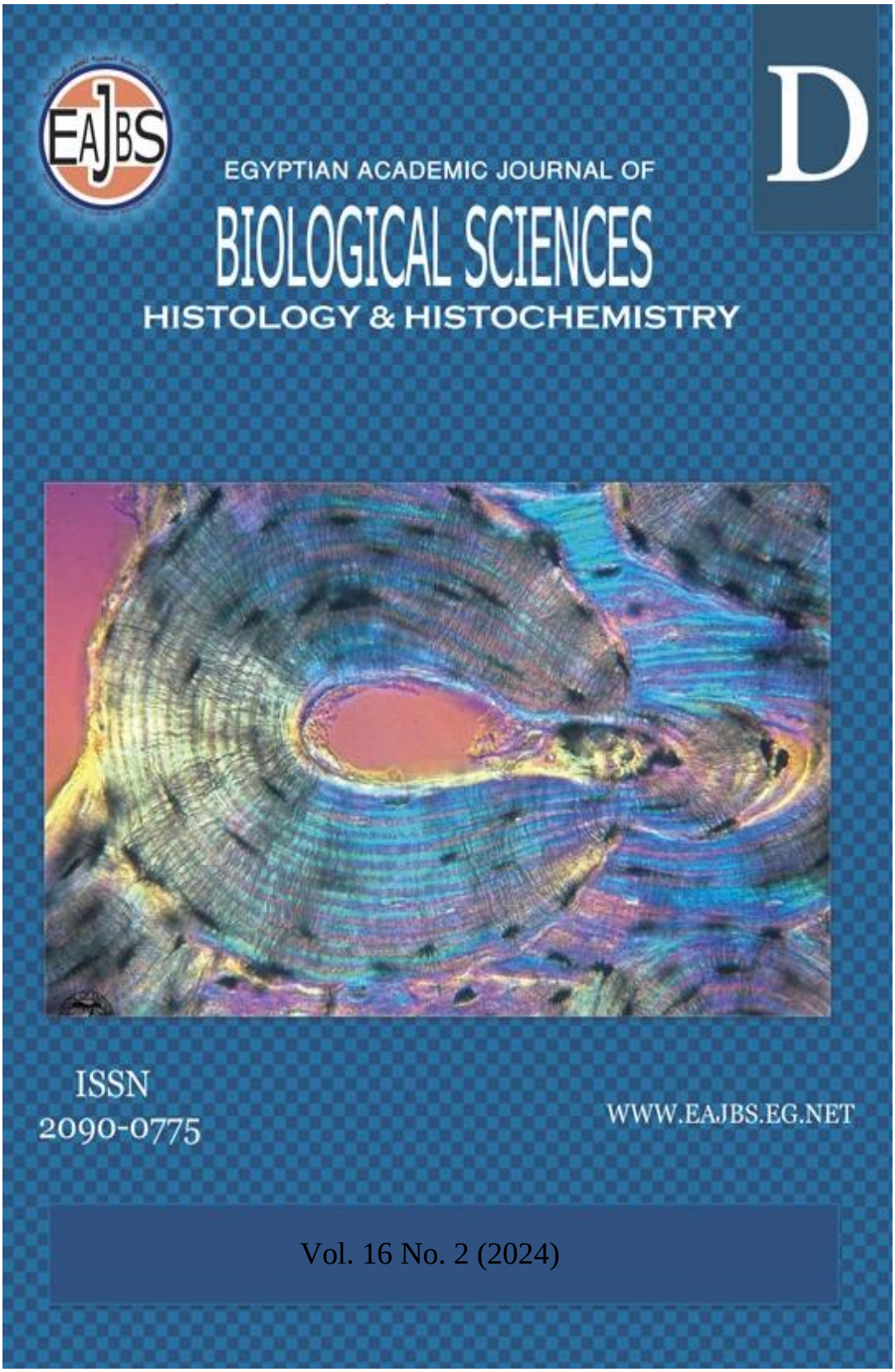

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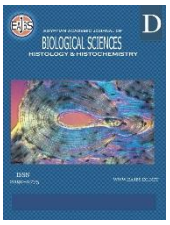

Bioaccumulation of Phenols, Oxidative Stress Biomarker Response and Histopathological Alterations in *Heterotis niloticus* (Cuvier, 1829) from Epe Lagoon, Lagos, Nigeria

Akeredolu Excellence[1], Egure M. Oizamsi [1], Ovioma G. Onoriode[2], Kuton M. Pentho[3], Johnson O. Deborah[1]; Isibor P. Omoregie[4], and Akinsanya Bamidele[1]

[1]Department of Zoology, University of Lagos, Akoka, Lagos, Nigeria.
[2]Department of Biological Sciences, Yaba College of Technology, Lagos.
[3]Department of Marine Sciences, University of Lagos, Akoka, Lagos, Nigeria.
[4]Department of Biological Sciences, Covenant University, PMB 1023, Ota, Ogun State.
*** E-mail** : arundhathi19@yahoo.co.in

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ABSTRACT

This study was aimed at analyzing the bioaccumulation of phenols, histopathological alterations, and oxidative stress biomarker response in *Heterotis niloticus* from Epe lagoon, Lagos, Nigeria. The study investigated the concentration of phenol in the intestine, liver and parasite of *Heterotis niloticus* fish in a Epe lagoon. Specimens of *H. niloticus* were freshly purchased randomly from the Olowu waterside market in Epe. The oxidative stress biomarker response was also carried out to examine the stress caused to *Heterotis niloticus* in Epe lagoon, on exposure to phenolic compounds. Also, physical and chemical parameters of the surface water where the fishes were collected were also analyzed. Most of the physicochemical parameter results showed no significant variations in relation to established limits, excluding Ph which was slightly acidic, and Dissolved Oxygen which exceeded the permissible limits. 2-Nitro-Phenol, 4-Chloro-3-methyl-Phenol, 2, 4, 6-Trichloro-Phenol and 4-Nitro-Phenol are the phenol compounds present in the intestine, 2-Nitro-Phenol, 2, 4, 6-Trichloro-Phenol and 4-Nitro-Phenol were found in the liver, while only 2-Nitro-Phenol and 4-Nitro-Phenol were found in the parasite of *Heterotis niloticus* in Epe lagoon, Lagos. The oxidative stress biomarker GPx was highest in the intestine, SOD was highest in the parasite, while MDA, GSH and CAT were significantly low in the intestine, liver and parasite of the fish. Significant histopathological alterations in the whole organisms were observed in the intestine of the fish samples. The pathological findings in the infected fish ranged from increase in the connective tissue of the submucosa, focal area of haemorrhagic lesion and loss of villous structure. The findings show how phenol pollution affects aquatic organisms and also reflects the poor health condition of the Epe lagoon, Lagos Nigeria.

INTRODUCTION

Phenol and phenolic compounds are universal toxicants that enter the natural water bodies from the effluents of a variety of chemical industries such as coal processing plants, phenol manufacturing, pharmaceuticals and industries of resin paint, dying, textile, wood, petrochemical, pulp mill (Moraes *et al.,* 2016). Industrial, domestic, agricultural and municipal activities constitute the anthropogenic sources of water pollution with phenolic compounds (Anku, 2017).

It has been observed that majority of these companies do not properly treat their effluents before releasing them into the aquatic environment since these water bodies serve as a sink for many industrial effluents. Lagos is situated in Nigeria's low-lying coastal region, which is characterized by rivers, creeks, lagoons, and estuaries. These water bodies are used for the disposal of waste from state-based companies. It is a rural community with a high concentration of people along the lagoon bank. The plant community of the lagoon is characterized by raphia palms and shrubs (Akinsanya *et al.*, 2015).

The effluents of numerous chemical industries, such as coal refineries, phenol manufacturing, pharmaceuticals, and resin, paint, dyeing, textile, leather, petrochemical, pulp mill, and other industries, introduce phenol and its compounds into natural water bodies (Mukherjee *et al.*, 1991, Fleeger *et al*. 2003). Phenols are water pollutants that can be found everywhere. Phenol and its compounds have been found to cause morphological, behavioral, and histopathological alterations in fishes such as African catfish *Clarias gariepinus* upon exposure with sublethal concentration of phenol (Ibrahem, 2011).

Heterotis niloticus, also known as African bony tongue is a large fish that is widespread in many tropical rivers and freshwater lakes of western and central parts of Africa (Moreau, 1982). It is the only species in the genus *Heterotis* and family *Osteoglossidae* (Agbugui *et al.*, 2000). This species of fish is widely distributed in freshwater bodies in Nigeria. The Juvenile and adult stage can be found in the Lagos lagoons. *H. niloticus* is usually consumed by majority of Nigerians because it is a rich source of protein with hardy flesh, and also tastes great. It is also highly valued because of its economic importance; a lot of fisher men rely on the catch and sales of this fish species as a source of income for their livelihood because it is normally large in size and can be sold for a high price. It is usually sold fresh, dried or smoked. In fertilized ponds, *H. niloticus* usually attains a singular mean weight of up to 3kg to 4 kg within a year (Monentcham *et al.,* 2009). This species has likewise been effectively introduced in many rivers and aquaculture stations in Africa (Monentcham *et al.*, 2009).

There is dearth of information on the bioaccumulation of phenol in *Heterotis niloticus*. This study will be beneficial to researchers, policy makers and also to regulatory bodies, to make informed decisions on the environmental impact of phenols on aquatic organisms, their habitat and also to protect the consumers of the aquatic organisms.

MATERIALS AND METHODS

1-Study Area:

The study area is in Epe lagoon, Lagos, Nigeria. Lagos is situated in Nigeria's low-lying coastal region, which is characterized by rivers, creeks, lagoons, and estuaries (Figure 1). The State's land mass is made up of 25% water (Ndimele, 2003). These water bodies are used for the disposal of waste from state-based industries (Anetekhai *et al.*,2007). The lagoon has a surface area of 243 km^2 and has been subjected to pollution since it is a sink for both industrial, agricultural and domestic waste. Most of the industries discharge priority pollutants such as phenol which have shown to accumulate in fish tissues (*Heterotis niloticus*-Africa bony tongue fish, *Chrysichthys nigrodigitatus*-catfish and Tilapiazilli-red tilapia).

The four main lagoons in Lagos state are Epe, Lekki, Lagos, and Ologe. Epe Lagoon lies between latitudes 6°29 N and 6°38 N; and longitudes 3°30 E and 4°05 E (Agboola and Anetekhai, 2008). The lagoon's maximum depth in a significant portion is roughly 6.0 meters. It is a rural community with a high concentration of people along the lagoon bank. The plant ecology surrounding the lagoon is made up of raphia palms and bushes. The

species of fish found in Epe lagoon include; *Heterotis niloticus, Gymnarchus niloticus, Clarias gariepinus, Malapterurus electricus, Synodontis clarias, Chrysichthys nigrodigitatus, Parachanna obscura, Mormyrus rume, Calabaricus calamoichthys, Tilapia zilli, Tilapia galilae, Hemichromis fasciatus* and *Sarotherodon melanotheron (Kusemiju,* 1981).

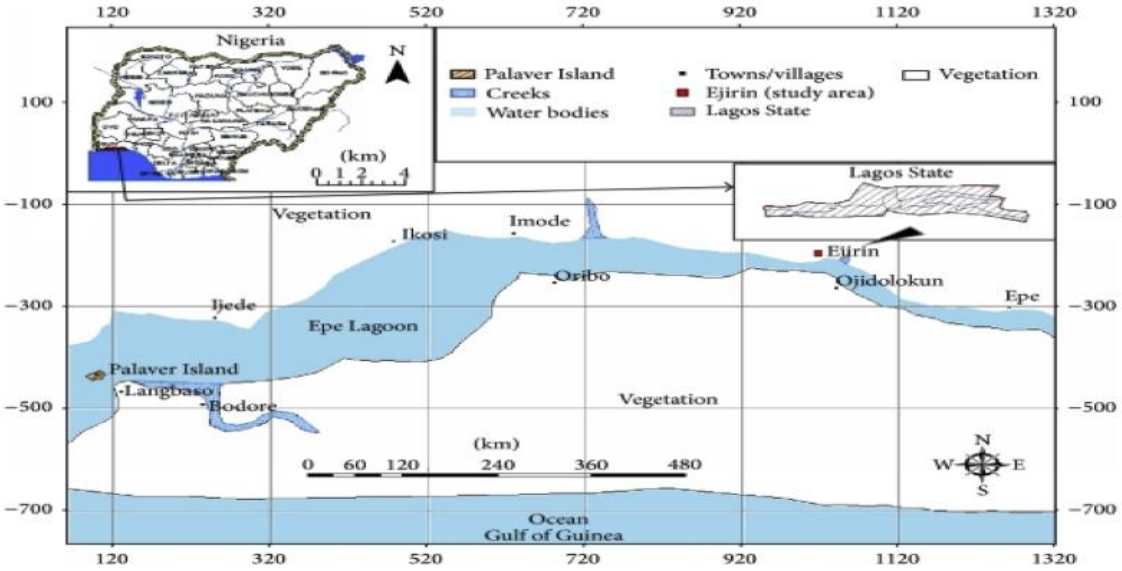


Fig. 1: Map showing study site and sample location

2-Extraction of Organic Fraction from Water Samples:

A 250 ml separatory funnel was filled with 100 ml of the water sample (or its equivalent ratio). A total of three extractions were made using 20 ml of methylene chloride and produced a final extracting solvent of about 60ml. For one to two minutes, the separatory funnel was covered and shaken rapidly while being regularly vented to let our extra pressure. At least 10 minutes were given for the organic layer to separate from the aqueous phase. This was done by decanting the organic layer into a fresh beaker or round-bottom flask. Prior to fractionation for phenols, the sample extracts (organic layer) were concentrated to about 1 to 2 mL using a rotary evaporator. About 1 to 3 spatulas worth of activated sodium sulphate were then added to the concentrated extract in order to remove water/aqueous portions.

3-Extraction of Organic Fraction from Sediment Samples:

10g ± 0.05g of homogenized sample was weighed into 250 mL Teflon bottle. The samples in the Teflon bottles were treated with about 1 to 3 spatulas worth of activated sodium sulphate to remove any water or aqueous components. Three extraction procedures using 20mL of 1:1 acetone: hexane resulted in a final extracting solvent of about 60 mL. The Teflon bottles were then sonicated for 30 minutes at 70°C in an ultrasonic bath. The organic layer was removed and decanted into a clean beaker or round-bottom flask. After additional drying with sodium sulphate, the silica gel column was used for clean-up. Before examination with a gas chromatography mass spectrometer, the sample extract was condensed to 2mL using a rotary evaporator (GC-MS).

4-Extraction from Biological Tissues:

Using KOH Refluxing/Vortex Extraction (Vassilaros *et al.,* 1982) – EPA Method 3611C (Clean up Method), 15g wet weight of tissue was weighed into a crucible, macerated, and then homogenized. 10g of the homogenized tissue was then placed in a 50mL centrifuge tube, 15mL of 6N KOH was added, and the tubes were sealed. The tubes were then incubated for 18 hours at 35^{o}C with vigorous shaking for 30 sec every 12 h for the first 4 h. The sample was allowed to cool. In order to facilitate

phase separation, 15 mL of methylene chloride or ethyl ether were added to the centrifuge tube, vortexed for 1 minute, and then centrifuged at 2000 rpm for 5 minutes. Using Pasteur pipettes, the upper/aliquot layer was extracted into a 250mL round-bottom flask. The solvent was centrifuged twice and all of the aliquot portions were combined in the round bottom flask. Prior to fractionated clean up using an alumina gel column and GC-MS analysis, sample extracts concentration to approximately 5–10mL was carried out using a rotary evaporator.

5-Determination of Phenols:

The stock was used to generate five (5) point serial dilution calibration standards (0.25, 0.50, 1.00, 2.00, and 4.00ppm), which were utilized to calibrate the GC-MS. The abundance of m/z 69, 219, and 502 as well as other instrument optimum & sensitivity conditions were checked using pre-established criteria to auto-tune the MS to perfluorotributylamine (PFTBA) prior to calibration. Using GC-MS, the levels of phenols in the sample were determined. To ensure low level detection of the target constituents, the MSD was operated in SIM and Scan modes. Agilent 7820A gas chromatograph coupled to 5975C inert mass spectrometer (with triple axis detector) with electron-impact source (Agilent Technologies) was used. HP-5 capillary column coated with 5% phenyl methyl siloxane (30m length x 0.32mm diameter x 0.25m film thickness) (Agilent Technologies) served as the stationary phase for the separation of the compounds. The carrier gas was Helium used at constant flow of 1.9 mL/min at an initial nominal pressure of 8.7712 psi and average velocity of 31.397 cm/sec at an initial temp of 40° C and holdup time of 1.5925 min.1µL of the samples were injected in a pulsed split less mode at an injection temperature of 220°C. With a total flow of 52.83 mL/min and a purge flow of 50.0 mL/min at 0.25 minutes, the gas saver mode flow was 20 ml/min after 2 minutes. Oven was originally set to 40 °C (2 min), then ramped at 40 °C to 100 °C (0.5 min), and finally to 140 °C (1 min). With a 3 min solvent delay and a 0.5 ml equilibration time, the run time was 33 minutes. The mass spectrometer was performed in electron-impact ionization mode with an electron energy of 70eV, an ion source temperature of 230 °C, a quadrupole temperature of 150 °C, and a transfer line temperature of 325 °C. Scan mode and selective ion mode were used to acquire the ions, which were scanned at a rate of 2.0 scans per second from m/z 50 to 500 amu (SIM). After calibration, the samples were examined, and the appropriate phenol concentration was determined.

6-Processing of intestine for Histopathological Analysis:

The infected and uninfected intestine were placed in separate bottles containing bouins fluid. After 6 h, the bouins fluid in each bottle was decanted. Then, 10% phosphate buffer formalin was added to preserve the tissue. Random selection was made from the preserved tissues based on single or multiple infections and light, heavy or no infection. The dehydration of the tissues took place in increasing concentrations of alcohol and twice in absolute alcohol at 30 min interval. Tissues were impregnated in molten paraffin three times and later embedded in molten paraffin wax and allowed to solidify. The blocked tissues were sectioned at 4-5 microns floated into pre-coated slides and dried. The sections were stained using haematoxylin and eosin stains. The stained tissues were washed off in tap water and over stained ones destained in 1% acid alcohol. The tissues were mounted using DPX mountant dried and examined under the microscope. The photomicrographs were taken in the pathology laboratory of the Department of Veterinary pathology, University of Ibadan, Nigeria.

8-Determination of Parasite Prevalence and Mean Intensity:

Examination of fish parasite was carried out using the techniques of Bich and Dawaki (2010), Omeji *et al.*, (2010), Akinsanya *et al.*, (2007) and

Emere and Egbe (2006).Each fish was extracted and the intestine of each fish was removed and placed in petri dishes containing 0.09% normal saline. Each intestine was carefully teased open from the anterior to the posterior end to aid the removal of the parasite from the intestine. The emergence of any parasite was seen by its wriggling movement in the saline solution. Some of the parasites however remained attached in the intestine with the aid of their attachment organ. These attached parasites were removed with the aid of forceps.

According to Ezewanji *et al.,* (2005), the parasitic prevalence and mean intensity were calculated using the formula below:

$$\textbf{Prevalence}\% = \frac{\text{Number of fish infected}}{\text{Number of fish examined}}$$

$$\textbf{Mean intensity} = \frac{\text{Total number of parasites}}{\text{Number of fish infected}}$$

$$\textbf{Abundance} = \frac{\text{Number of collected parasites}}{\text{Number of fish examined}}$$

$$\textbf{Bio-load} = \frac{\text{Number of collected parasites}}{\text{Number of fish infected}}$$

8-Calculation of Bioaccumulation Factor (BAF):

The transfer factor in fish tissues from the aquatic ecosystem was calculated according to Kalfakakour and Akrida-Demertzi (2000) and Rashed (2001) as shown below:

$$\text{TF} = \frac{\text{Metal concentration in intestine}}{\text{Metal concentration in water}}$$

9-Antioxidant Enzymes Sampling:

Oxidative enzymes were assayed in the liver, intestine and parasite of the fish samples. The fishes were dissected and the livers, intestine and parasite were collected into different labeled sampling bottles.

9.1-Determination of Glutathione (GSH):

The reduced glutathione content of the tissues and parasite were estimated according to the method described by Jollow *et al.*, 1974. 10% of Trichloroacetic acid (TCA) was added to the tissues homogenate and then centrifuged for 10 minutes at 25^0c and 4000Rpm. After centrifugation, 0.5ml of the supernatant was taken in a test tube and treated with 0.25ml of Ellman's reagent (9.9mg of 5, 5-dithiobisnitro benzoic acid (DTNB) IN 100ml of 0.1% sodium nitrate) and 2.2 ml of phosphate buffer. The absorbance was read at 412 nm.

9.2-Catalase (CAT) Antioxidant Activity Assay:

This was assayed by the method of Aebi, (1974). In the CAT assay, 0.4ml of H_2O_2 was taken with a micropipette and added to 0.1ml of the supernatant sample homogenate. The change in absorbance was read spectrophotometrically at 240 nm. Phosphate buffer was used to blank (remove air) when taking the reading using the OPTIMA SP-3000 PLUS spectrophotometer.

9.3-Malodialdehyde (MDA):

Malondialdehyde (MDA), a final product of polyunsaturated fatty acids peroxidation was determined using the method of Buege and Aust (1978). 0.1ml of sample was added to 2ml of TCA-TBA-HCl reagent (Thiobarbituric acid 0.37%, 15% of TCA and 0.25m HCl) in the ratio of 1:1:1. The mixture of sample and TCA-TBA-HCl reagent was boiled for 15 minutes, allowed to cool and then centrifuged at 3000 rpm for 10 minutes. The supernatant was carefully removed with a dropper and put in a cuvette. The absorbance of the supernatant was read with a spectrophotometer at 535nm against a blank MDA.

9.4-Determination of Superoxide Dismutase (SOD) Activity:

According to Sun and Zigma(1978), the ability of superoxide dismutase to prevent the autooxidation of epinephrine was measured by an increase in absorbance at 480 nm. The reaction mixture (3ml) contained 2.95ml, 0.05M sodium carbonate buffer pH 10.2, 0.02ml of liver homogenates and 0.03ml of substrate (epinephrine) and 0.02ml of water. Enzyme activities were determined by

measuring the variation in absorbance at 480 nm for 5 minutes.

9.5. Determination of Total Protein (Biuret Method):

Pipette 1.0ml of the biuret reagent (test) and 1.0ml of the reagent blank (reagent blank) into test tubes for every sample. 20µl of water was poured to the blank and 0.02 µl of each sample was added to the test. A standard test tube is also set up for each batch, containing 20 µl of a standard protein and 1 ml of the biuret reagent. After mixing, allow the mixture rest for 30 min at room temperature or 10 min at 37 ^{o}C. To zero the instrument, use the reagent blank solution. Calculate the absorbance of the test and the standard at a 546 nm wavelength.

10-Statistical Method:

Analysis of variance (ANOVA), SPSS IBM, 20.0 was used to compare means of Phenols concentrations in the Livers, tissues and parasite of *Heterotis niloticus* in Epe lagoon. Microsoft excel 2007 was used for the graphs.

RESULTS

1-Physicochemical Characteristics of the Epe Lagoon:

The lagoon has a mean pH of 6.631 with standard deviation of 2.834, mean temperature at 31.19^{0}C with standard deviation of 2.834 (Table 1). The lagoon has a mean salinity of 0.771ppt, turbidity of 2.147 NTU and dissolved oxygen of 5.750 mg/l with standard deviation of 0.552, 1.086, and 0.726 respectively. The electrical conductivity of 0.516 µs/cm, total dissolved solids of 0.329g/L and dissolved oxygen of 5.750% with mean standard deviation of 0.046, 0.006, and 0.726 respectively.

Table 1: Physicochemical Parameters of Epe Lagoon

Parameters	Mean	Standard deviation
Temperature	31.19	2.834
Ph	6.631	0.156
Conductivity	0.516	0.046
Turbidity (NTU)	2.147	1.086
Dissolved oxygen (m/l)	5.750	0.726
Total Dissolved Solid	0.329	0.006
Salinity (ppt)	0.771	0.552

2-Bioaccumulation of Phenol in liver and intestine of *Heterotis niloticus* from Epe Lagoon:

Table 2, shows the different concentrations of phenol componenets in the liver, intestine and parasite of *Heterotis niloticus.* The concentration of the total phenol was higher in the intestine than in the liver.Chloro-3-Methyl-Phenol was higher in the intestine with mean value of 5.09±0.004mg/kg and lower in the liver, 2.63±1.38mg/kg and lowest in the parasite with mean value of 1.09±1.70mg/kg. 2-Nitro-Phenol was high in intestine, 3.29±0.003mg/kg and low liver, 1.19±0.62mg/kg and lower in the parasite. The concentration of 2,4,6-Trichloro-Phenol in the intestine, liver and parasite of *Heterotis niloticus* were 2.04±3.29mg/kg, 0.00±0.00mg/kg and 0.00±0.00mg/kg respectively.The concentration of 4-Nitro-Phenol in the intestine was 1.49±0.001mg/kg, liver 0.00±0.00mg/kg and parasite 0.00±0.00mg/kg. 2-Chloro-Phenol, 0-Cresol (2-Methyl-Phenol), 2,4-Dimethyl-Phenol, 2,4-Dichloro-Phenol, and Pentachloro-Phenol in the intestine, liver and parasite were below threshold limits with concentrations of 0.00±0.00mg/kg respectively.

The concentration of Phenol in the fish was significantly higher in the intestine as compared to the liver and parasite.

Table 2: Concentration of phenol components in the liver, intestine and parasite of *Heterotis niloticus.*

Phenol Components	Intestine	Liver	Parasite	Sig Value
	Mean±SD	Mean±SD	Mean±SD	0.00
4-Chloro-3-Methyl-Phenol	5.09±0.004^{c}	2.63±1.38^{b}	1.09±1.70^{a}	0.00
2-Nitro-Phenol	3.29±0.003^{c}	1.19±0.62^{b}	0.62±0.69^{a}	0.068
2,4,6- Trichloro-Phenol	2.04±3.29^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.00
4-Nitro-Phenol	1.49±0.001^{b}	0.00±0.00^{a}	0.00±0.00^{a}	-
Phenol	0.00±0.00	0.00±0.00	0.00±0.00	-
2-Chloro-Phenol	0.00±0.00	0.00±0.00	0.00±0.00	-
0-Cresol (2-Methyl-Phenol)	0.00±0.00	0.00±0.00	0.00±0.00	-
2,4-Dimethyl-Phenol	0.00±0.00	0.00±0.00	0.00±0.00	-
2,4-Dichloro-Phenol	0.00±0.00	0.00±0.00	0.00±0.00	-
Pentachloro-Phenol	0.00±0.00	0.00±0.00	0.00±0.00	-

Mean values with different superscripts are significantly different (p value <0.05)

3-Bioaccumulation factor in the intestine and liver of *H. niloticus* from Epe Lagoon:

Phenol congeners were accumulated in the intestine and liver and this was used to determine accumulation factor (BAF) and presented in figures 2 and 3. Only 2-Nitro-Phenol and 4-Nitro-Phenol showed significant BAF in the intestine of the fish.

Table 3: Bioaccumulation Factor in the intestine of *H. niloticus* from Epe Lagoon

Phenol Components	BAF
2-Nitro-Phenol	0.2157
4-Nitro-Phenol	0.1884
2,4,6-Trichlorophenol	0
4-Chloro-3-Methyl-Phenol	0
Phenol	0
2-Chloro-Phenol	0
0-Cresol (2-Methyl-Phenol)	0
2,4-Dimethyl-Phenol	0
2,4-Dichloro-Phenol	0
Pentachloro-Phenol	0

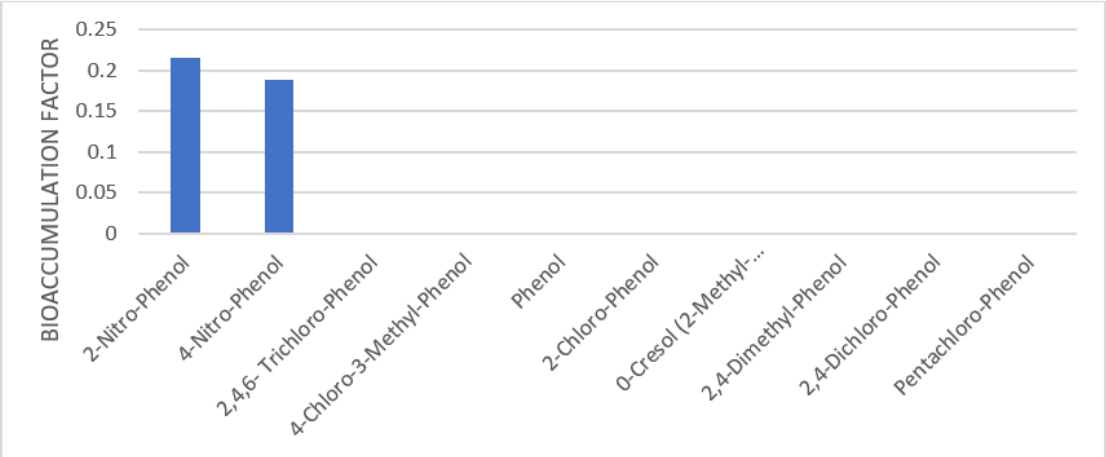


Fig. 2: Bioaccumulation Factor of Phenol in intestine of *H. niloticus* from Epe Lagoon

Table 4: Biaccumulation factor in the Liver of *H. niloticus* from Epe Lagoon

Phenol Components	BAF
2-Nitro-Phenol	0.416712655
4-Nitro-Phenol	0.508508844
2,4,6-Trichlorophenol	0
4-Chloro-3-Methyl-Phenol	0
Phenol	0
2-Chloro-Phenol	0
0-Cresol (2-Methyl-Phenol)	0
2,4-Dimethyl-Phenol	0
2,4-Dichloro-Phenol	0
Pentachloro-Phenol	0

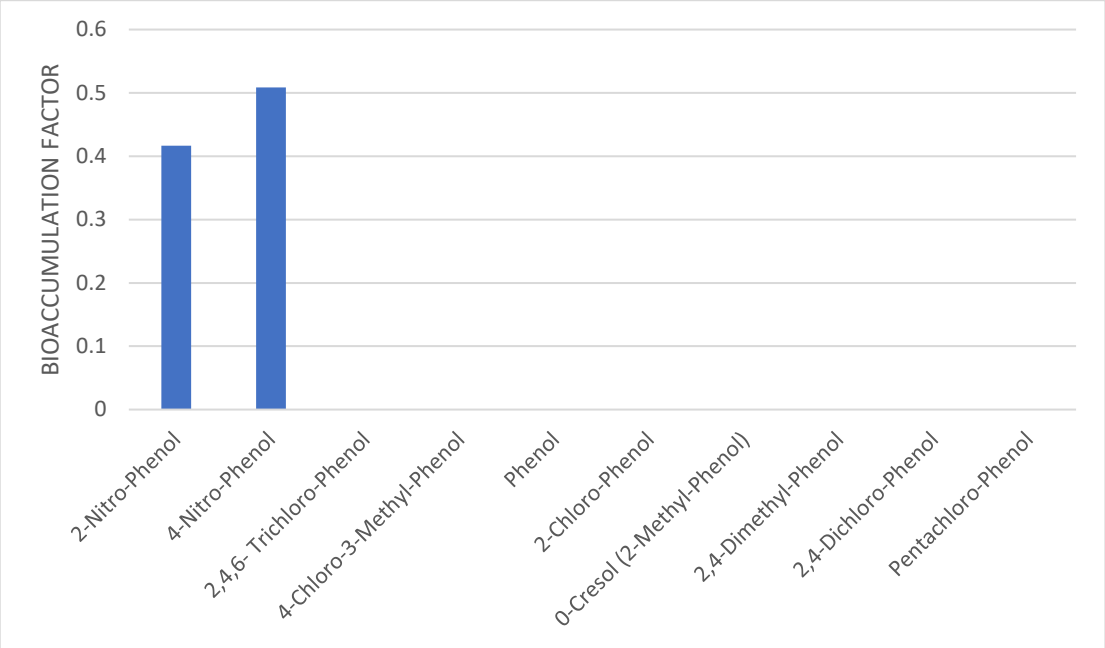


Fig. 3: Bioaccumulation Factor in the Liver of *H. niloticus* from Epe Lagoon

4-Oxidative Stress Biomarkers in *H. niloticus* from Epe Lagoon:

Table 5, shows the oxidative stress biomarker response in *H. niloticus* from Epe lagoon. The oxidative stress response parameters; superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidase (GPX), protein (PRO) and lipid peroxidation (Malondialdehyde) in the liver and intestine. In the Intestine, the decreasing order of mean value are as follow, GPX (699.6±46.26)> SOD (355.6±72.76)> CAT (3.501±0.730)> MDA (3.328±1.128)> GSH (3.053±0.881). In the Liver, the highest to the lowest mean value are as follow, GPX (864.5±375.6)> SOD (330±84.19)> GSH (4.126±0.928)> MDA (3.146±1.163)> CAT (2.953±0.801). In the Parasite, the highest to the lowest mean value are as follow, GPX (618.5±324.2)> SOD (398.9±54.70)> MDA (3.973±1.102)> CAT (2.981±1.201)> GSH (1.958±0.664).

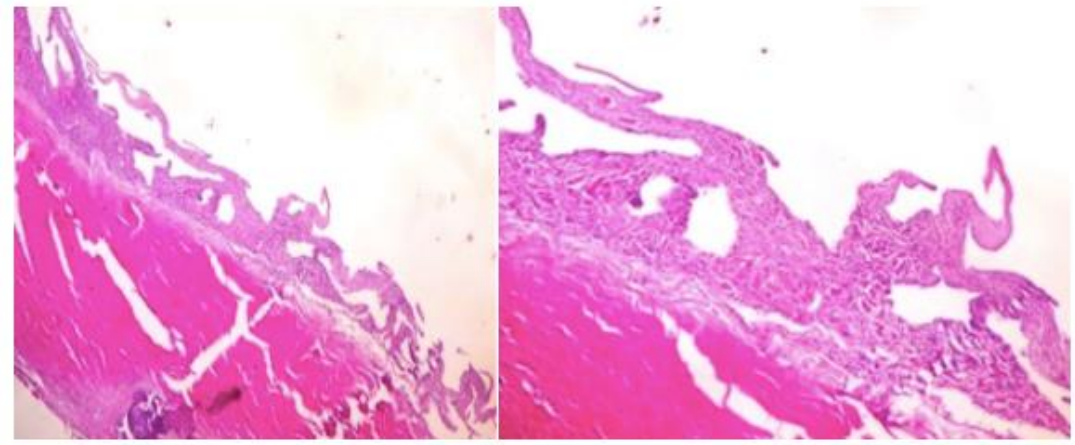
Table 5: Oxidative stress biomarker response in *H. niloticus* from Epe Lagoon, Lagos, Nigeria

Enzymes	Intestine		Liver		Parasite		Sig Value
	Mean	SD	Mean	SD	Mean	SD	
CAT	3.501[a]	0.730	2.953[a]	0.801	2.981[a]	1.201	0.328
SOD	355.6[a]	72.76	330.4[a]	84.19	398.9[a]	54.70	0.268
MDA	3.328[a]	1.128	3.146[a]	1.163	3.973[a]	1.102	0.421
GSH	3.053[b]	0.881	4.126[c]	0.928	1.958[a]	0.664	0.001
GPx	699.6[a]	46.26	864.5[a]	375.6	618.5[a]	324.2	0.251

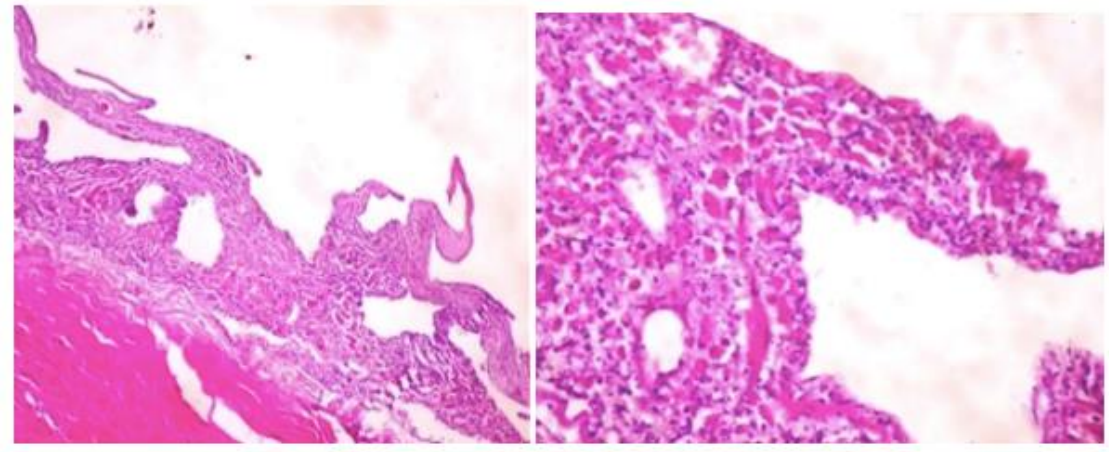
Mean values with different superscripts are significantly different (p value <0.05)

5-Histopathological Alteration Index in The Itestine of *Heterotis niloticus* from Epe Lagoon

The intestine had mild stunting of villi and loss of intestinal gland, increase in the connective tissue of the submucosa, focal area of haemorrhagic lesion and loss of villous structure (Plates 1&2).



H&E X40 H&E X10



H&E X400 H&E X400

Plate 1: Photomicrographs of intestinal tissue show normal villi structure, normal mucosa, submucosa and muscularis. The normal crypt-villous architecture is well preserved

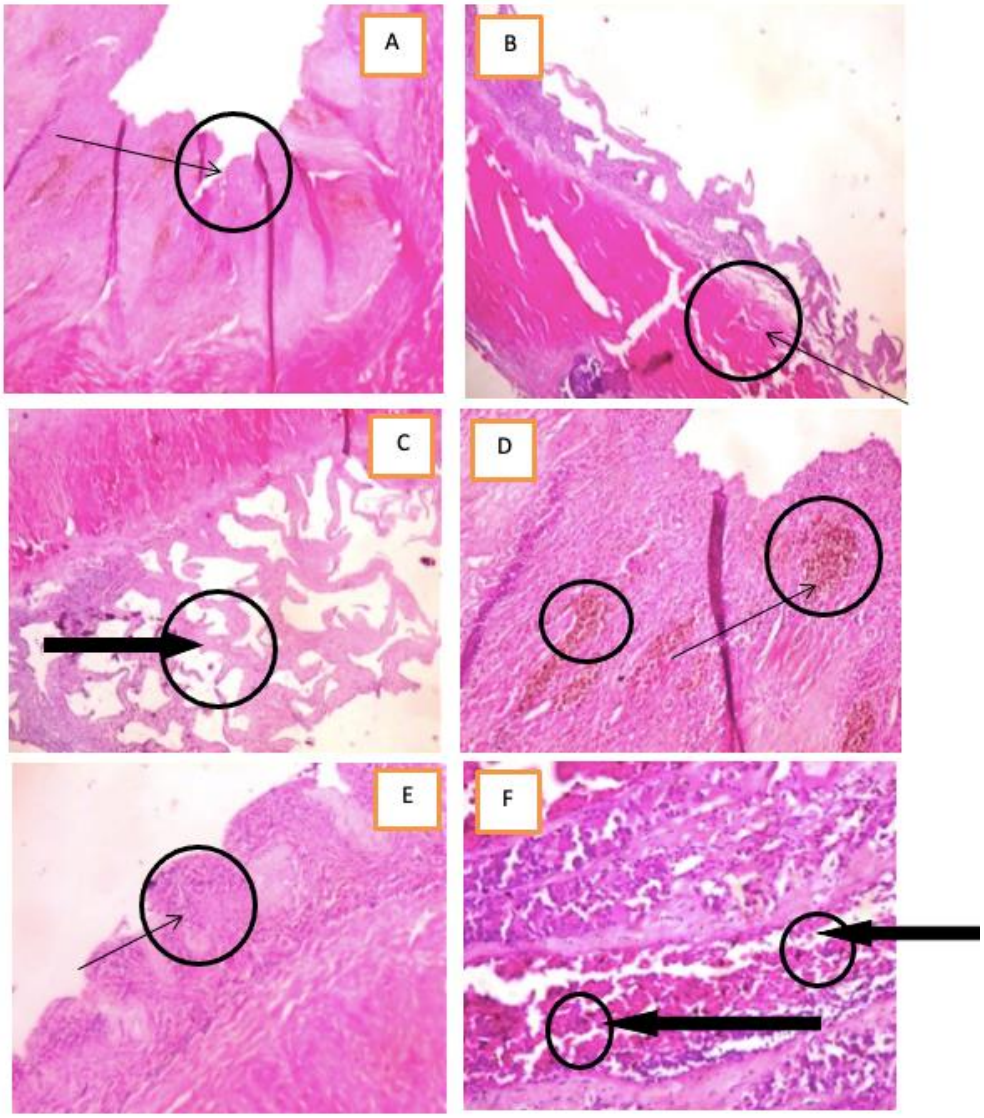


Plate 2: Photomicrographs of intestinal tissue show a marked increase in the connective tissue of the submucosa (black arrow), focal area of haemorrhagic lesion and loss of villous structure (slender arrow)**.**

DISCUSSION

The results of this study have expounded on the status of the Epe Lagoon in relation to phenolic compound pollution and the health condition of the African bony tongue fish, *Heterotis niloticus*.

The examination of the water body's surface water physicochemical parameters revealed no significant variations in most of the parameters measured in relation to the prescribed limitations (Federal Ministry of Environment). The recorded values fell within the FMenv on-spec range, except for pH with mean value of 6.631 .. The Electrical conductivity has a mean value of 0.516 which is within on-spec as compared to the FMenv allowable limit of 10,000. Also, the Total Dissolved Solids (TDS) was within permissible limits with a mean value of 0.329 which is within range for the FMenv permissible limits of 500. The concentration of dissolved oxygen is directly linked with the amount of oxygen produced by photosynthesis and consumed by living things, particularly bacteria (Aniyikaiye *et al.*, 2019). The low Dissolved oxygen in the Epe lagoon may be as a result of excessive nutrient load (nitrogen and phosphorus) from the release of produced waters either through point or non-point sources into the water body (Xiuqin *et al.*, 2020), this can lead to excessive algae growth

(Eutrophication). The algae depletes the oxygen content of the water body, thereby resulting in hypoxia (He *et al.*, 2014; Li *et al.*, 2014). Hypoxia condition in water body can lead to the death of fishes in the water body (Domenici *et al.*, 2017). This condition harms can cause harm to the aquatic ecosystem and also have negative impact on fishermen who depend on these resources for their livelihood and customers who anticipate plentiful fish harvest. The pH of 6.631 indicates that the water body is slightly acidic. This slightly acidic condition may increase the bio availability of toxic chemicals already present in the lagoon (Akinsanya *et al.*, 2020).

Majority of the fishes were found to be infested by parasites especially in the intestine. The results also showed that four phenol compounds were discovered in the intestine namely: 2-Nitro-Phenol, 4-Chloro-3-methyl-Phenol, 2, 4, 6-Trichloro-Phenol and 4-Nitro-Phenol while two phenol compounds: 2-Nitro-Phenol and 4-Chloro-3-methyl-Phenol were present in the liver and parasite. Based on comparison, the total amount of phenol compounds accumulated in the intestine is higher than in the liver and parasite. This is because the liver is primarily involved in fish physiology, detoxification, metabolism, and immune defense (Kime *et al.*,1998, Nemeth *et al.*, 2009). The parasites possibly had significant sequestration functions to play in releasing the host fish from the burden caused by the chemical (Akinsanya *et al.*, 2020). The presence of the phenol compounds in the intestine and liver of the fish in this study shows that they are threatened by endocrine disrupting compounds. It is reported that the presence of some phenol compounds such as; bisphenol-A (BPA), nonylphenol 4-NP and octylphenol 4-t-OP in aquatic ecosystems act as endocrine disrupting compounds (Olujimi *et al.*,2012, LV *et al.*, 2019, Ibor *et al.*,2022).

MDA, SOD, CAT, and GPx are frequently used as outstanding biomarkers for assessing oxidant/ antioxidant status, and the activity of antioxidant enzymes varies depending on the length and intensity of applied stress (Qu *et al.*, 2015). MDA is the most common byproduct of the oxidation of polyunsaturated fatty acids, and its level is utilized as an indicator for lipid peroxidation (Mukherjee *et al.*, 2016). Superoxide radicals (O^{2-}) produced in mitochondria and peroxisomes are converted to hydrogen peroxides by the enzyme SOD, which provides protection against damage caused by free radicals. The enzyme CAT then eliminates the hydrogen peroxide from the body by converting it to water (H_2O) and molecular oxygen (O_2). This study is in line with that of (Bassey, 2019), who found that the liver and muscle of *Chrysichthys nigrodigitatus* fish had considerably higher mean SOD activity. The degradation of an environmental site by lowering the water quality can be linked to an increase in MDA levels in organisms (Bassey, 2019). This report shows that there are high levels of SOD and GPX in the intestine, liver and parasite of the fish,

The significant increase in GPX and SOD activities observed in intestine, liver and parasite may represent an adaptive response to protect the fish from free radical toxicity induced by phenolic compounds. Research on Phenolic compounds concentration in the environment has received much attention by environmental toxicologist, and regulatory agencies due to their toxic, carcinogenic and persistent properties (Anku, 2017, and Zuzana, 2011, Moraes *et al.*, 2016). Phenolic compounds can accumulate in the tissues and organs of living organisms. Histopathology is an important method used in the assessment of toxic substances in aquatic organisms from polluted ecosystems. This helps to provide opportunity to detect and locate the effect of pollutants in various organs and tissues of organisms. The Histopathological alteration shows some effects on the intestine

Conclusion

The current values of Epe Lagoon's physical and chemical parameters revealed that Temperature, Conductivity, Total Dissolved Solids and Turbidity were within FMenv safe standards while pH and Dissolved oxygen were over those limits. The health evaluation of the fish in the lagoon showed the presence of some components of phenol in the tissues ad parasite which may pose threat to the fish. Evaluation of physicochemical analysis only might not accurately reflect the actual biological state of the aquatic ecosystem. The parasite showed significant elevation in the Oxidative stress biomarker response compared to the intestine, indicating that they help in sharing the load burden in the intestine of the fish, since the parasites reside in the intestine of the fish. The study suggested that the fish may have antioxidant marker activity in different body tissues under stress conditions, such as on exposure to phenolic compounds or other toxic chemicals. This study also suggests shows that phenol can accumulate in the tissues of *Heterotis niloticus*, which can cause stress to the fish, and in turn, pose a risk to human health. As a result of this, the Epe lagoon should be monitored on a regular basis.

Since the rate of industrialization around the EPE lagoon has continuously been on the increase, Regulators should make use of baseline data provided by researchers to make more stringent laws regarding the release of produced water and other pollutants into the water body. Regulators should also ensure the enforcement of these laws by carrying out regular auditing of the industries' equipment and systems as this will make industries comply to the laws and regulations regarding the release of harmful waste into the water body.

Declarations:

Ethics Approval: Institutional (The Gujarat Cancer and Research Institute) approval was taken. (IRC/23/2019 dated 14/11/2019).

Conflict of Interest: The authors declare no conflict of interest.

Author contribution: The designed of this work was conceptualized by some of the authors while others also joined to actualize the research. Other authors also joined in the writing of the manuscript.

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