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EFFECT OF RED TASSEL (*Emilia sonchifolia*) FLOWER ON THE HISTOLOGY OF *Clarias gariepinus* FINGERLINGS

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Abstract

Medicinal plants can boost the immunity of fish, but can also become harmful if the toxicity level goes beyond safe limit in aquatic environments. The present study investigated the effect of *Emilia sonchifolia* flower on the histology of *Clarias gariepinus* fingerlings. A 96 hours' static bioassay was used throughout the study. 10 fishes were stocked in each aquarium, in triplicates, in 6 groups, totaling 18 aquaria. Histological results after 96 hour's exposure of *C. gariepinus* to different concentrations of *E. sonchifolia* leaf powder shows negative alterations on the gills, kidneys, livers and gonads when compared with the control groups. Temperature and pH significantly increased, while dissolved oxygen significantly decreased in the exposed groups compared with the control. Results from this study shows that medicinal plants can become toxic to fish at certain concentrations. Therefore, their level of abundance in aquatic environment should be regulated.

Keywords: *Emilia sonchifolia*, *Clarias gariepinus*, Histology

1.0 Introduction

Emilia sonchifolia is a plant used in traditional medicine to treat several viral bacterial diseases (Chen *et al.,* 2009). It is able to do so because of the phytochemicals it possesses that included alkaloids, tannins, flavonoids and sterols (Lamai *et al.,* 2015). Phytochemicals are the make-up of plants; they determine the extent in which plants express their curative or toxicity tendencies. *Emilia sonchifolia* has been reported to have shown anti-oxidants, anti-inflammatory, anti-cancer and anti-tumor activities (Yadava & Manta, 2012). In aquaculture, fish diseases have been implicated as one of the major causes of fish kills; (Bahmani *et al.,* 2001). A single outbreak of Edwardsiellosis almost wiped out a whole population of Carp, Eels, Channel catfish, Tilapia and Stripped bass (Hassan *et al.,* 2010). The young fishes are usually the most affected because of their fragile immune systems. Parasite invasion into the physiological systems of fish has caused loses in both private and commercial fisheries establishment, which can be linked to lack of standard sanitary facilities in the sector.

Clarias gariepinus, though reported by several authors (Kashimuddin*et al.,* 2021, Prokesova*et al.,* 2015 &Alexandrova*et al.,*

2021) as hardy fish species, that endures harsh condition that includes: poor management and feeding, also have been affected by several infectious diseases, that causes deleterious effect on their histology. El-Moselly *et al.,* (2014) reported that bio-accumulation of metals by fish is deposited in different organs such as gills, kidney, heart, liver, brain, digestive tract and bones. Copper for example, is an important micro nutrient for aquatic organisms, but can become the most dangerous to them when accumulated in their organs.

Emilia sonchifolia is one of the abundant plant species commonly found around aquatic environments. By being present around aquatic environments, they are submerged in the water, which brings about loss of leaves nutrients via leaching, microbial colonization and fragmentation of leaves by micro invertebrates (Webster and Benfield, 1986), thereby having fishes come in contact with the plant, by way of making the plant a part of their diet for herbivores and omnivores in the food chain. It is therefore, safe to infer that fish and other aquatic organisms will enjoy some level of immunity against infectious diseases, considering the medicinal potentials of *Emilia sonchifolia* composite flower. This study seeks to determine the medicinal and by extension, the toxicity potential of *Emilia sonchifolia*flower on *Clarias gariepinus* fingerlings.

2.0 Methodology

2.1 Location of the study

This study was carried out at the wet laboratory of the Department of Fisheries and Aquatic Science, University of Cross River State (UNICROSS), Faculty of Agriculture and Forestry. Obubra campus.

2.2 Research materials

The research materials included *Clarias gariepinus* fingerlings of mean weight 22.5±0.3 grams and length 9.25±0.4 centimeters, *Emilia sonchifolia* plant leaves powder, water quality parameters kits (thermometer; EZ-9910, dissolved oxygen; DO-9100 and pH meter; CRP-100), 18 plastic aquaria, sensitive weighing balance, dissecting kits, organ holding containers, 10 percent formaldehydes, and laboratory experimental tables.

2.3 Experimental procedures

Four hundred *Clarias gariepinus* fingerlings of mean weight 22.5±0.3 grams and length 9.25±0.4 centimeters were procured from Standard Ford Fish Farm, Ikom Local Government Area of Cross River State. The fish were transported at around 06:30 am to 08:00 am from the fish farm to the experimental site. They were acclimated for a fortnight, and were fed five percent of their total body weight with 0.8 mm coppens feed. The *Emilia sonchifolia* (Red Tassle) plant was harvested around the University of Cross River State (UNICROSS) fish farm.

The plant was authenticated by the use of a plant atlas. They were harvested, cleaned, air dried for two weeks and then pulverized with a laboratory mortar and pestle, and adequately sieved with 0.4 mm laboratory net, and stored at room temperature for the experiment thereafter. The method according to Gololo *et al.,* (2017) was used. In this method, the pulverized leaves powders were put directly into the water containing the experimental fish.

Prior to the expiration of acclimation, 18 plastic aquaria were washed and air dried, disinfected and labelled T0, T1, T2, T3 T4 and T5 in triplicates, where T0 was the control and T1-T5 represented different concentrations of *Emilia sonchifolia* plant leaves powder. Twenty liters of de-chlorinated tap water were measured into each aquarium, and 10 fishes were randomly distributed into each of the aquarium (Sunday

et al., 2018), using a scoop net. The experiment to investigate the effect of the plant leaves powder on the histology of *Clarias gariepinus* fingerlings lasted for 96 hours (acute toxicity test).

2.4 Water quality parameters

2.4.1 Temperature

The water temperature was checked at intervals, during the experiments, using mercury in glass thermometer. The temperature meter EZ-9910 was inserted inside of the aquarium for about three minutes and removed thereafter to read and record the values indicated on the mercury.

2.4.2 Dissolved Oxygen Concentration

A dissolved oxygen meter, model DO-9100, was used to measure this electronically and to the nearest 0.1 mg/L. After being turned on, calibrated, and having the probe submerged in the water for one minute, the dissolved oxygen meter's stable readings were taken and recorded.

2.4.3 pH

The pH was also measured using the electronic method, model CRP-100. This is carried out by simply turning on the meter, and the probe is inserted into the water after calibration to the nearest 0.0 mg/L and the vales are read off and recorded accordingly.

2.5 Histopathology

At the end of the 96-hour experiments, a fish was taken from each tank for histopathological studies. The sampled fish were carefully dissected with sharp knife and forceps from a dissecting kit to collect the gills, kidneys, livers and gonads. The organs were stored inside disinfected containers, containing 10 percent formaldehyde, and immediately transported to the laboratory for histological investigation, according to Akin-Obasola (2019). The procedures used for the analysis involved: tissue processing: dehydration using ascending grades of alcohol (70 percent, 90 percent and three changes of absolute alcohol), clearing, using two changes of xylene, infiltration using molten paraffin wax, embedding, microtomy, haematoxylin and eosin staining, dewax and hydrate, rinse in water, stain in haemaroxylin, rinse with water, dye in eosin, blue in tap water, rinse with water, drench, clear, and mount, microscopy and imaging were carried out with a high definition Leica microscope (Leica D 750), interpretation – images were analyzed and interpreted accordingly.

2.6 Statistical analysis

Data for water quality was collated and analyzed using one-way analysis of variance. The differences in mean between values were assessed with Duncan Multiple Range Test, using SPSS version 18.0 at $p<0.05$ significant level (Frank &Althoen, 1995). Histological results are presented pictorially.

3.0 Results

3.1 Water quality

When compared to the control, the parameters indicating the water quality varied significantly amongst the groups. Values for water quality parameters are displayed in Table(one). While means with distinct superscripts are statistically different, those with the same superscripts are statistically the same.

3.1.1 Temperature

The definitive experiments showed that *Emilia sonchifolia* leaves powder had effect on the temperature of the water. The temperature from the control with average values of 25.50^0C rose to 30.03^0C in some of the exposed groups in the experiment. It was seen that increase in temperature was time and concentration related (one).

3.1.2 Dissolved oxygen

The dissolved oxygen showed a significant decrease in the exposed groups, with increase in the concentration of *Emilia sonchifolia*as

seen in table (one). This indicated that *E. sonchifolia* leaves powder had effect on the dissolved oxygen.

3.1.3 pH

The pH values obtained showed a significant decrease in exposed groups, with increase in time and concentration of *Emilia sonchifolia* leaves powder; indicating that the pH values responded to the effect of *E. sonchifolia* as seen in table (one).

3.2 Histological parameters

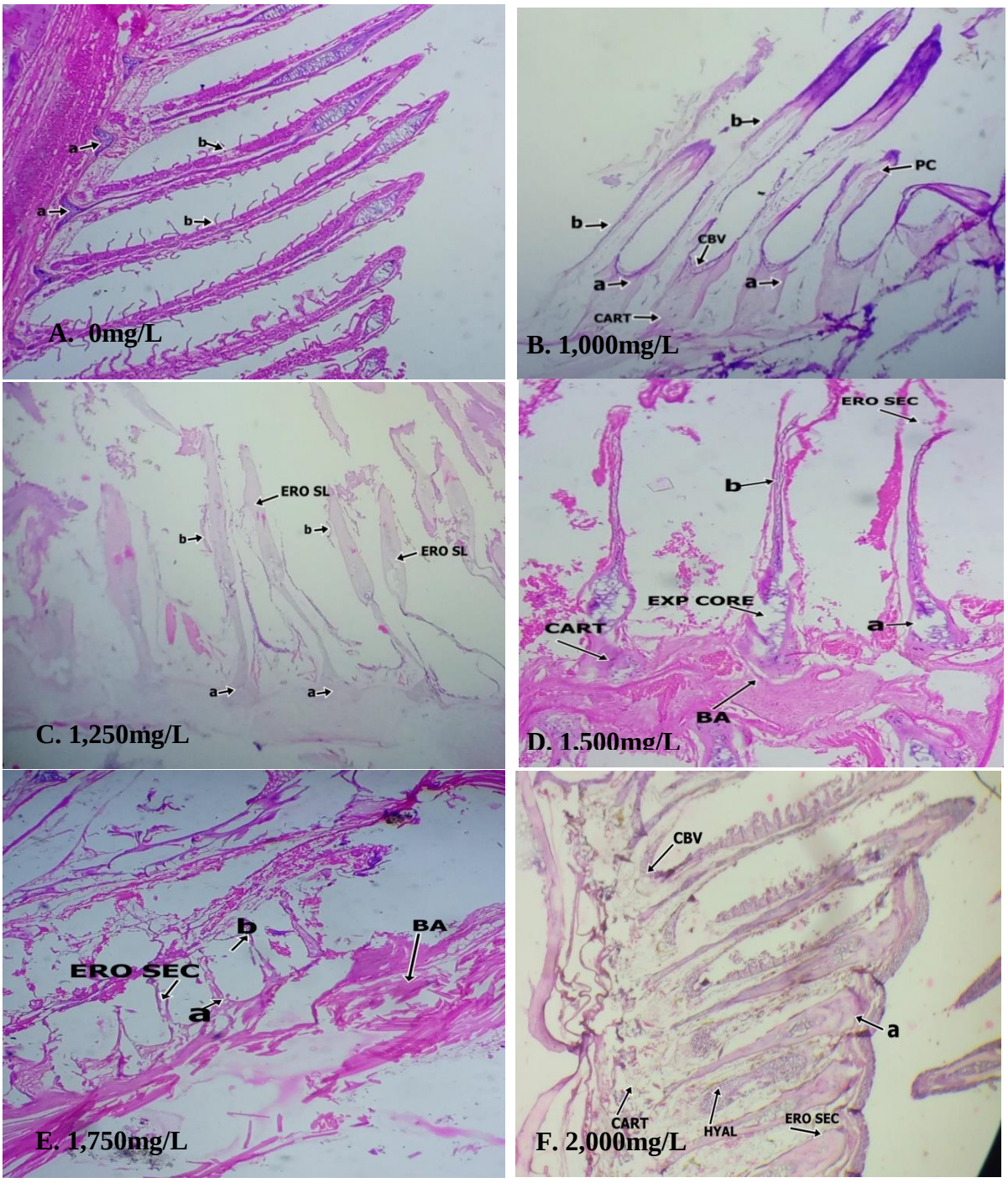
The results presented from plates one - four (A-F) represents the histological changes observed in the fingerlings of *Clarias gariepinus* exposed to *Emilia sonchifolia* leaves powder. Histological changes in the organs were observed in all the exposed groups, although different concentrations gave different results. The normal histology of the gills structure of *C. gariepinus*fingerlings exposed to 0 mg/L of *E. sonchifolia* leaves powder is seen in plate one (A).

TABLE 1: Summary of water quality parameters of *Emilia sonchifolia* powder on *Clariasgaripienus*fingerings

	Parameters	0mg/L	1,000mg/L	1,250mg/L	1,500mg/L	1,750mg/L	2,000mg/L
BEFORE	Temp. (°C)	25.53±0.3^{a}	25.52±0.3^{a}	25.40±0.3^{a}	25.44±0.3^{a}	25.53±0.3^{a}	25.55±0.3^{a}
	$Do_{2\ (mg/L)}$	6.24±0.1^{a}	6.27±0.1^{a}	6.28±0.1^{a}	6.27±0.1^{a}	6.30±0.1^{a}	6.28±0.1^{a}
	pH	6.29±0.1^{a}	6.42±0.1^{a}	6.39±0.1^{a}	6.37±0.1^{a}	6.32±0.1^{a}	6.34±0.1^{a}
24HRS	Temp. (°C)	26.36±0.2^{a}	26.47±0.2^{a}	27.52±0.2^{b}	27.72±0.2^{b}	28.51±0.2^{c}	28.73±0.2
	$Do_{2\ (mg/L)}$	6.68±0.1^{b}	4.83±0.1^{a}	4.77±0.1^{a}	4.76±0.1^{a}	4.63±0.1^{a}	4.60±0.1^{a}
	pH	6.76±0.1^{b}	6.74±0.1^{b}	6.75±0.1^{b}	6.74±0.1^{b}	6.63±0.1^{b}	6.42±0.1^{a}
48HRS	Temp. (°C)	27.31±0.2^{a}	27.34±0.2^{a}	27.36±0.2^{a}	28.23±0.2^{b}	28.86±0.2^{b}	28.87±0.2^{b}
	$Do_{2\ (mg/L)}$	6.43±0.2^{c}	4.76±0.2^{b}	4.43±0.2^{a}	4.42±0.2^{a}	4.32±0.2^{a}	4.21±0.2^{a}
	pH	6.73±0.1^{b}	6.68±0.1^{b}	6.62±0.1^{b}	6.61±0.1^{b}	6.47±0.1^{a}	6.43±0.1^{a}
72HRS	Temp. (°C)	27.59±0.3^{a}	27.94±0.3^{a}	28.26±0.3^{b}	28.73±0.3^{b}	28.94±0.3^{b}	29.04±0.3^{c}
	$Do_{2\ (mg/L)}$	5.88±0.2^{c}	4.09±0.2^{b}	4.01±0.2^{b}	3.97±0.2^{a}	3.96±0.2^{a}	3.96±0.2^{a}
	pH	6.73±0.1^{b}	6.63±0.1^{b}	6.32±0.1^{a}	6.21±0.1^{a}	6.02±0.1^{a}	6.02±0.1^{a}
96HRS	Temp. (°C)	27.84±0.2^{a}	28.30±0.2^{b}	28.32±0.2^{b}	29.22±0.2^{c}	29.76±0.2^{c}	30.03±0.2^{d}
	$Do_{2\ (mg/L)}$	5.62±0.1^{c}	4.02±0.1^{b}	4.00±0.1^{b}	3.52±0.1^{a}	3.42±0.1^{a}	3.32±0.1^{a}
AFTER	pH	6.76±0.1^{c}	6.53±0.1^{c}	6.52±0.1^{c}	6.22±0.1^{b}	6.01±0.1^{b}	5.99±0.1^{a}

Means with the same superscript in the row are not significantly different ($p>0.05$)

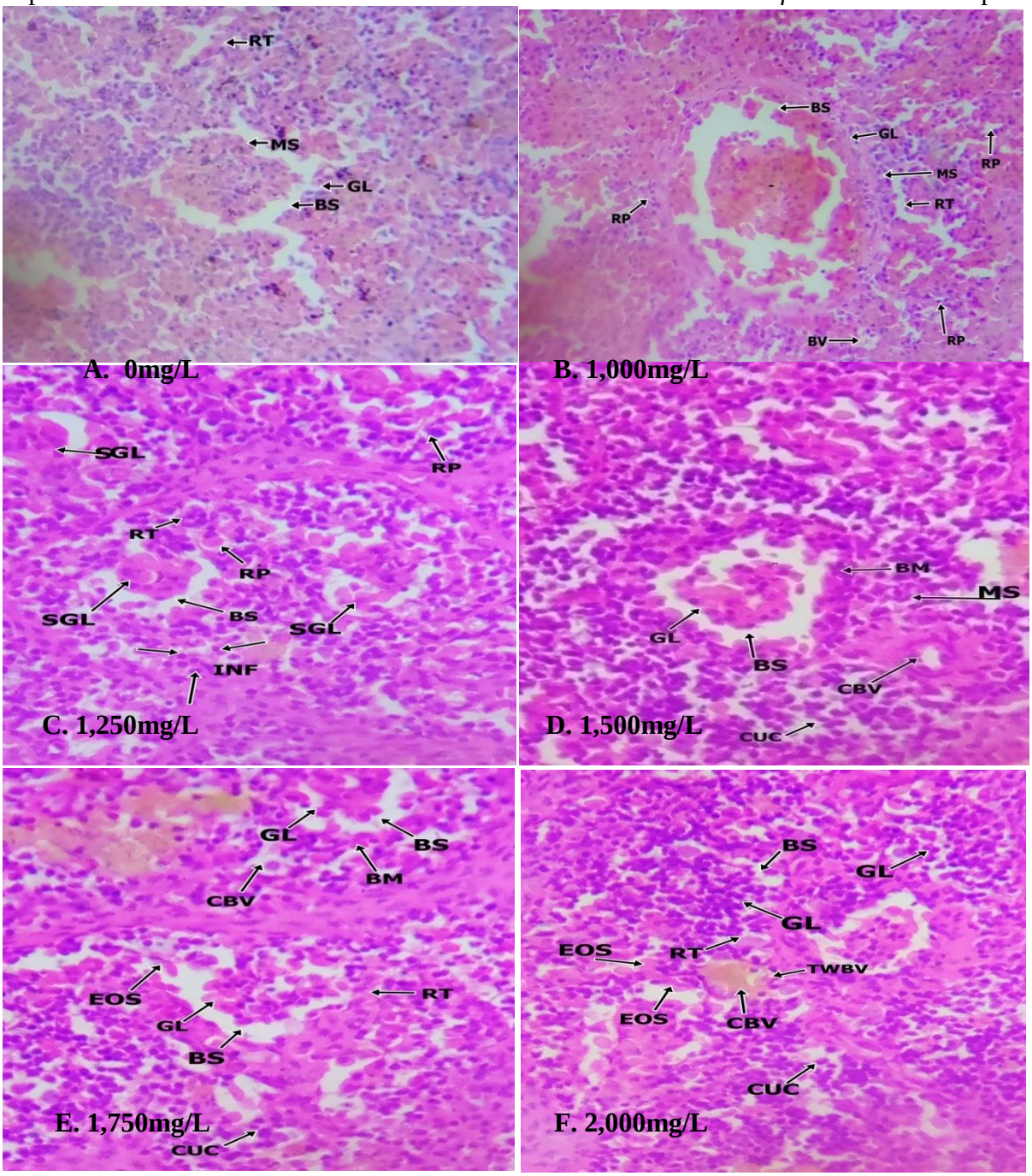
Plate 1: (A-F). Histological changes observed in the gills of *Clarias gariepinus*fingerlings exposed to different concentrations of *Emilia sonchifolia* leaves powder.



A. Normal tissue structure, no pathological lesion observed. B. Dilated primary lamellae, and congested blood vessels. C. Eroded and less distinct secondary lamellae. D. Eroded lining of the epitheliums. E. Degeneration of the secondary lamellae. F. Congested blood vessels, eroded epithelium lining, and absence of secondary lamellae.

(a – Primary lamellae, b – Secondary lamellae, ERO SL – Eroded secondary lamellae, BA – Bony arch, ERO SEC – Eroded surface epithelial cells, CART – Cartilage, HYAL – Hyaline, CBV – Congested blood vessels, PC – Proliferating cells).

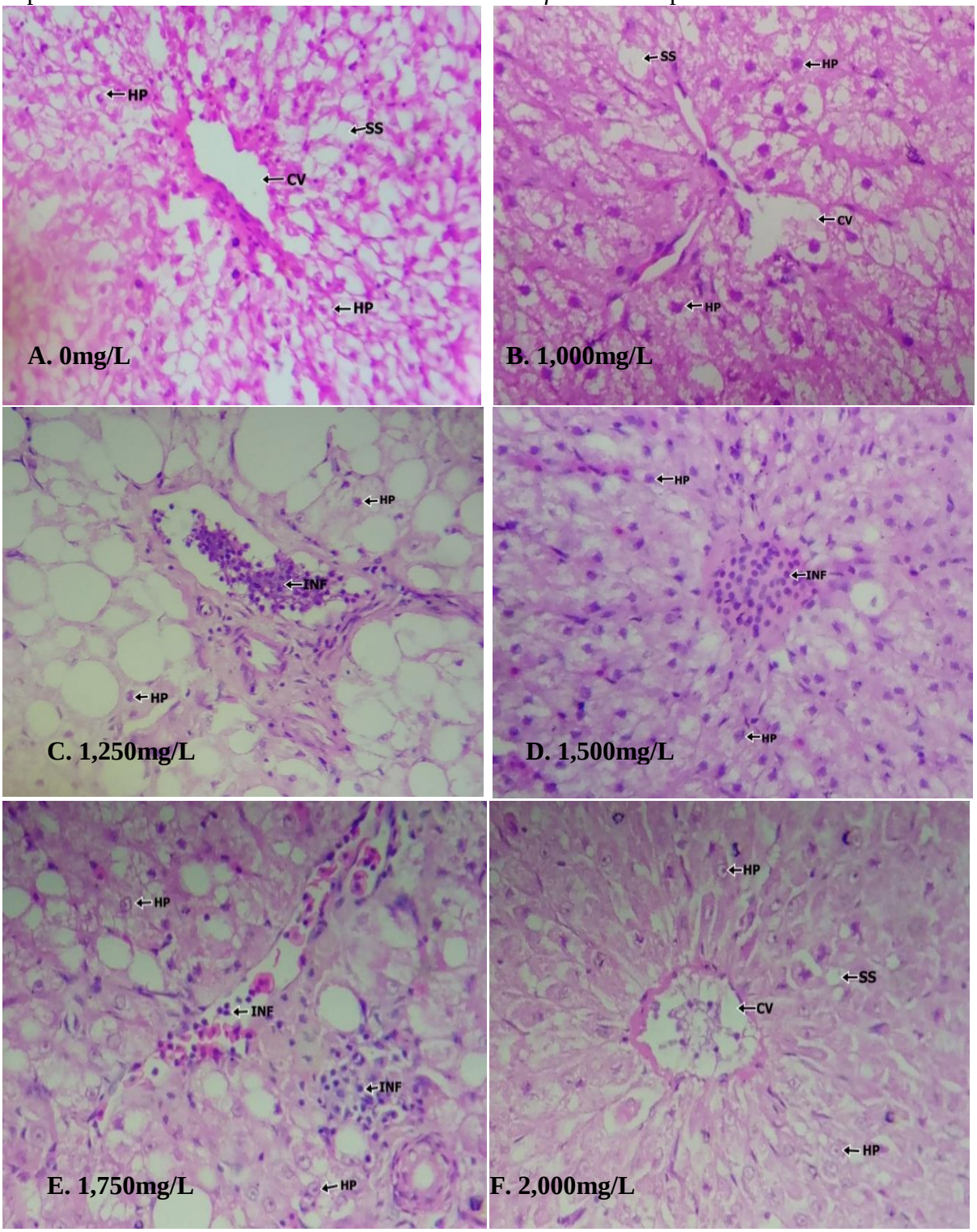
Plate 2: (A-F). Histological changes observed in the Kidneys of *Clarias gariepinus*fingerlings exposed to different concentrations of *Emilia sonchifolia* leaves powder.



A. Normal kidney layer, no pathological lesion observed. B. Normal kidney layer, no pathological lesion observed. C. Swollen glomerulus, and scanty inflammatory cells. D. Congested blood vessels. E. Congested blood vessels and scanty eosinophilic secretion. F. Thin walled blood vessels, and scanty eosinophilic secretion.

(RT –Renal Tubules, MS – Mesangium, GL – Glomerulus, BS – Bowman space, BV – Blood vessels, SGL – Swollen glomerulus, INF – Inflammatory cells, EOS – Eosinophilic secretion, TWBV – Thin walled blood vessels, CUC - Cuboidal).

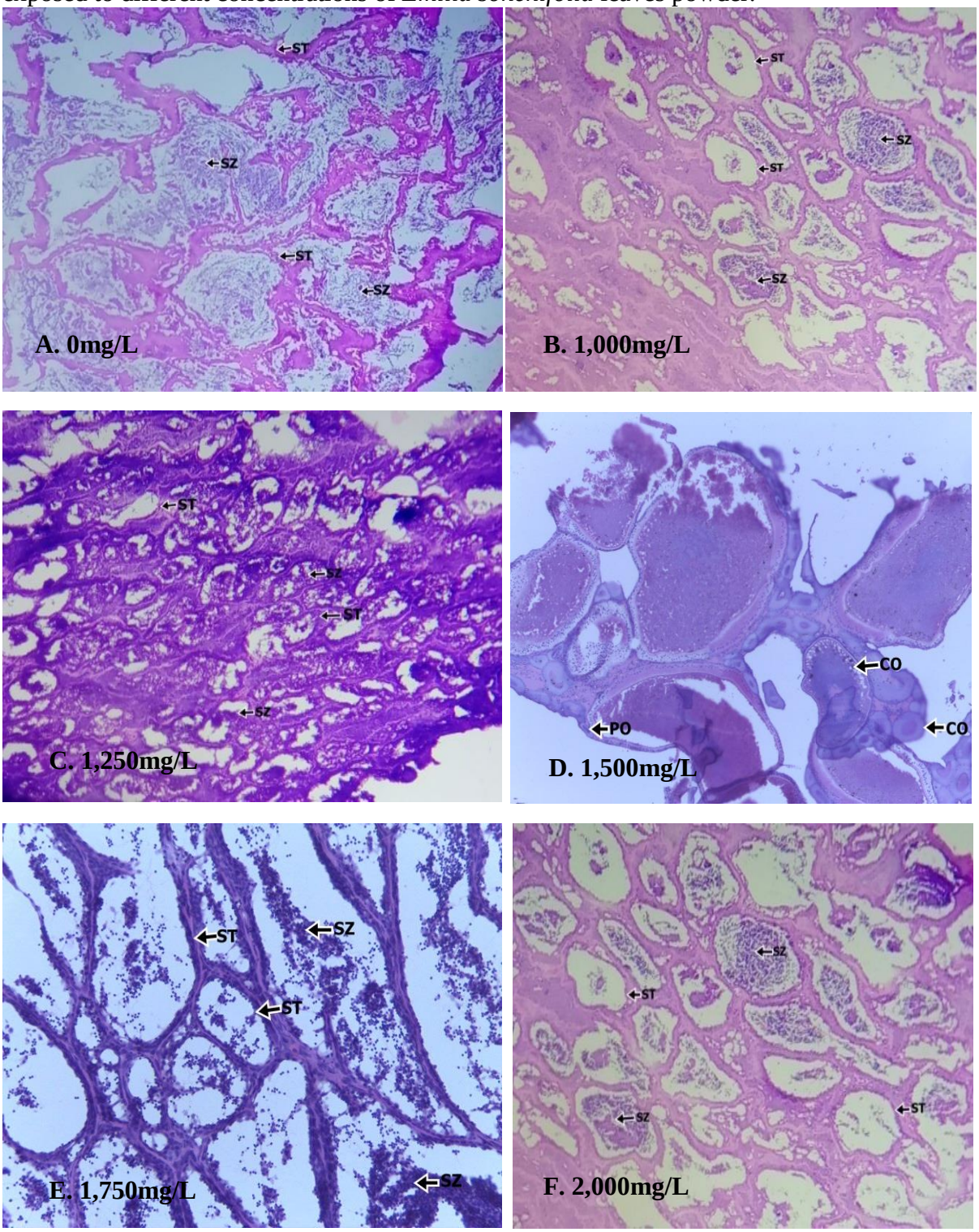
Plate 3: (A-F). Histological changes observed in the Livers of *Clarias gariepinus*fingerlings exposed to different concentrations of *Emilia sonchifolia* leaves powder.



A. Normal liver tissue, no pathological lesion observed. B. Normal liver tissue, no pathological lesion observed. C. Fatty degeneration. D. Moderate amount of mononuclear inflammatory infiltrates. E. Severe fatty degeneration and moderate cellular injury. F. Complete degenerated cells.

(HP – Hepatocytes, PT – Portal area, BV – Blood vessels, SS – Sinusoidal space, INF - Inflammation).

Plate 4: (A-F). Histological changes observed in the Gonads of *Clarias gariepinus* fingerlings exposed to different concentrations of *Emilia sonchifolia* leaves powder.



A. Normal gonad tissue, no pathological lesion observed. B. Scanty population of germ cells. C. Scanty spermatocytes, spermatids and spermatozoa. D. Ovarian stroma. E. Scanty population of germ cells at various stage of maturation. F. Scanty spermatids.

SZ – Spermatozoa, ST – Spermatid, CO - Cortical Alveolar Oocytes, PO - Perinucleolar Oocytes. Differences in histological structures of the gills were observed to be the following: congested blood vessels in the primary lamellae plate one (B) at 1,000 mg/L of the plant leaves powder, eroded secondary lamellae (C) at 1,250 mg/L and eroded surface epithelial cells in (D, E and F) exposed to 1,500 mg/L, 1,750 mg/L and 2,000 mg/L of the plant leaves powder respectively.

The normal histology of the kidney structure of *C. gariepinus* fingerlings exposed to 0 mg/L of *E. sonchifolia* leaves powder is seen presented in plate two (A). Degeneration of the organs are recorded to be: swollen glomeruli, congested arterioles and scanty eosinophilic secretion across groups exposed to 1,000 mg/L, 1,250 mg/L, 1,500 mg/L, 1,750 mg/L and 2,000 mg/L of *E. sonchifolia* leaves powder, as seen in (B, C, D, E and F).

The normal histology of the liver structure of *C. gariepinus* fingerlings exposed to 0 mg/L and 1,000 mg/L of *E. sonchifolia* leaves powder are presented in plate three (A and B). Severe fatty degeneration as a result of mononuclear inflammatory infiltrates are presented in (C, D, E and F) at 1,250 mg/L, 1,500 mg/L 1,750 mg/L and 2,000 g/L of *E. sonchifolia* leaves powder.

The normal histology of the gonads structure of *C. gariepinus* fingerlings exposed to 0 mg/L of *E. sonchifolia* leaves powder are presented in plate four (A). Degenerations of the testes were reported as: scanty population of germ cells at various stages of maturation in (B and E), and also, scanty spermatid and spermatozoa in (C, D and F).

4.0 Discussion

The present study shows that *Emilia sonchifolia* leaves powder adversely affected the water quality parameters. Temperature in the groups exposed to different concentration of *E. sonchifolia*leaf powder were significantly higher (30.03^0 C) than those in the control(25.53^0C). This agrees with Alberho *et al.,* (2005), who reported that increase in leaf decomposition increases water temperature. Leaf decomposition in water occurs rapidly as a result of leaching of secondary compounds by microbial activities. However, temperature was not influenced when Fernanda *et al.,* (2016) carried out a study on the ‘’effect of leaf decomposition stage and water temperature on fragmentation activities of shredder invertebrates’ species in lotic ecosystem’ ’Dissolved oxygen most often than not expresses an inverse relationship with temperature. The dissolved oxygen in the present study significantly reduced from 6.24 mg/L to 3.32 mg/L when compared with the control. Increased metabolic activities as a result of the increase in temperature may have caused the significant decrease in the dissolved oxygen. Xu *et al.,* (2022) opined that dissolved oxygen of 2 – 3 mg/L causes stress in many fish species, significant reduction in growth and reproduction, and increase susceptibility to diseases as can be seen in the histopathological alterations in the gills and testes of the exposed fish to *E. sonchifolia* leaf powder. The pH level of the groups exposed to different concentrations of *E. sonchifolia* decreased significantly, when compared with the control. However, the significant decrease in the pH were within the recommended levels for teleost fishes (Reddy &Rawat, 2013).

The histopathological findings of this study demonstrated that the leaf powder had influence on the selected organs (gills, kidney, liver and testes) of *Clarias gariepinus* fingerlings. The gills are an integral part of fish, and they serve as an organ of biological homeostasis in the aquatic environment. The fish gills are critical because, they are organs for gaseous exchange; and as such, make it the closest to the external environment. They make the first organ to contend with xenobiotics in the aquatic environment (Prathepa & Sukumaran, 2014 &Mazon*et al.,* 2002). The present study showed that the condition of the gills in the control is not different from that of other teleosts, having no alteration.

However, the exposed groups showed alterations such as: eroded secondary lamellae and epithelium lining. This study agrees withIdowu *et al.,* (2019) that recorded gills alteration with increase in the concentration of *Euphorbia hirta* leaf extracts in *C. gariepinus*. The present study also, reported that alteration in the gills was dose dependent. Changes in gill morphologies may also have been as a result of interference of respiratory activities within the inter-lamellae spaces (water ways), which directly affects respiration across the gills (Reddy &Waskale, 2013).

The significant pathological index seen in the kidneys of the exposed fish as presented in plate 2 (C - D), in the present study were mainly swollen glomeruli, congested blood vessels, scanty inflammatory cells, bowman cells, interstitium and eosinophilic secretion, indicating the toxicity level of *Emilia sonchifolia* leaves powder to the kidney of *C. gariepinus*fingerlings, even at the lowest concentration. The above alterations are also indicative of the cells lacking necessary arsenals to combat the said toxicants, when exposed to the toxicants. This was also reported in the study of Idowu*et al.,* (2019) where *C. gariepinus* were exposed to *E. hirta* leaves extracts. The present study however contradicts Marina *et al.,* (2022) who rather reported hemorrhage and glomeruli shrinkage when *C. gariepinus* were exposed to *Malaleuca cajuputi* extracts. Shivappa (2015) also reported a contrary view to the present study where they discovered no alterations in the kidney, liver and heart of clown fish and gold fish, after exposure to *M. cajuputi*extracts (Melafix).

The liver is a bio transformational organ against xenobiotics (Dominic, *et al.,* 2014). In the present study, the control showed a preserved architecture consisting of intact hepatocytes, central vein, sinusoidal spaces, portal areas and bile duct, devoid of any fatty inflammation or degeneration. The exposed groups however, showed disorganized plates of hepatocytes radiating from dilated and congested central vein, macrovesicular and microvesicular steotosis, and cellular injury as presented in plate three (C - F). These observations agree with Kumar *et al.,* (2010) that stated that liver is one of the most sensitive organs that respond swiftly to pollutants attack in aquatic ecosystems. These may have been as a result of inflammatory infiltrates from *Emilia sonchifolia* leaf powder into the liver cells. The present study however disagreed with that of Dominic *et al.,* (2014) who exposed male Wister rats to Azaserine, to determine the effect of *E. sonchifolia*. They recorded a significant remedy in the liver and pancreatic enzymes upon the administration of hexane extracts of *E. sonchifolia*. This, they attributed to the therapeutic properties of the plant extracts, and especially because of the presence of terpernoids. Again, differences seen in the effect of the plant leaves powderin the liver functions of fish and rats may be as the result of differences in the metabolic, anatomy and physiological processes in Pisces and mammals.

The result of the gonads of *Clarias gariepinus* fingerlings in this study showed scanty spermatids and inadequate population of germ cells at various stage of maturation, as against the control that showed dense population of spermatogonia, spermatocytes, spermatid and moderate number of spermatozoa, as presented in plate four (B - F). This infers that *Emilia sonchifolia* leaves powder influenced the gonads. The degree of damage on the gonads of the test organisms may be the presence of steroids and flavonoids present in *E. sonchifolia* leaves powder. The steroids and flavonoids are well known endocrine disruptive chemicals. Phytosteroids that containsβ-sitosterol were reported to have disrupted the reproduction system of zebra fish; *Dainorerio* (Nakari&Erkomaa 2003). According to them, a three generations study exposing

zebrafish to wood sterol (containing β-sitosterol 80% β-sitosterol) and soy sterol (containing 50 % β-sitosterol) at concentration 10 and 20 µg L^{-1} for the former and 10 µg L^{-1} was carried out. The both sterols caused vitellogenin induction in the test organisms, while those exposed to wood sterol caused a change in sex ratio of the fish (Nakari&Erkomaa 2003). The first generation were predominantly male while the second were predominantly female, as against the intended mixed sex population. Bennetau-Pelissero *et al.,* (2001) found a decrease in spermatogenesis and sperm motility when genistein, an isoflavone with a well-known influence on gametogenesis and reproduction efficacy, was added to rainbow trout diet.

5.0 Conclusion and recommendations

Emilia sonchifolia adversely affected the histology (gills, kidneys, livers and testes) of *Clarias gariepinus* fingerlings at 1,000 mg/L – 2,000 mg/L, as recorded in the present study. It also negatively influenced the observed water quality parameters (temperature, dissolved oxygen and pH). However, the safe concentration limits of *E. sonchifolia* for *C. gariepinus* fingerlings, as recorded in this study are: 20 mg/L – 200 mg/L in 24 hours, 17.5 mg/L – 175 mg/L in 48 hours, 15 mg/L – 150 mg/L in 72 hours and 12.5 mg/L – 125 mg/L in 96 hours. We therefore, recommend that only plants and trees with low toxicity levels and high beneficial tendencies should be allowed within and around fish farms, routine water quality parameters should be carried out on pond waters, and fish farmers are also encouraged to carryout histological analysis on their fishes every once in a while, before commercialization.

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