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Haematological and Histopathological Responses of Catfish *Clarias gariepinus* Juvenile to Coconut Water *Cocos nucifera*

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Abstract
The toxicity of Coconut water *Cocos nucifera* L. (Arecaceae) water was determined on the Juvenile Catfish *Clarias gariepinus*. The 20, 24, 36, 48, 72 and 96LC_{50} of Coconut water to juvenile catfish were 750mg/l, 650mg/l, 550mg/l, 450mg/l, 350mg/l, and 250mg/l. While the total mortality occurred at a concentration of 750mg/l within 24hours exposure period respectively. Haematological examination showed an increase in blood cell count, and Platelet while there was a decrease in Haematocrit, MCV, MCH, LYMH, HMB, RBC in higher concentration of 750mg/l throughout the test in juvenile catfish *Clarias gariepinus*. There was a decrease in moisture, ether extract, nitrogen free extract and energy, from 73.78 ±10.73, 3.76 ± 0.34, 0.50 ± 0.08 in the control to 68.13 ±0.38, 3.17 ± 0.09, 0.37 ± 0.06 and 1.39 ±1.41, in higher concentration of 750mg/l, also there was an increase in Ash and Protein, from 0.6 ± 0.54 and 27.01 ±0.42mg/l in the control to 0.84 ± 0.14 and 26.28 ± 0.92mg/l in the concentration of 350mg/l. In the plasma electrolyte of catfish juvenile to coconut water, there was an increase in sodium from 1.06± 4.83 in the control to 74.54 ±26.09 in the concentration of 750mg/l, while in potassium; there was a decrease from 40.54 ± 3.43 in the control to 24.14 ±10.34 in higher concentration of 750mg/l. The result of histopathological responses of skin, gill and liver of the test organisms to coconut water showed different level of degeneration of cells, hypertrophy of gill arch, disarrangement of hepatic cell, necrosis, vacuolation, sign of trauma the gill, detachment of lamella, vessels congested with blood vessels, liver hypoxia, degeneration of the upper dermis and severe thinning of the underlying granular layer. Damages became severs with increase in concentration of aqueous extract and time exposure. The result of the tests provided the baseline information and established safe limits of using coconut water in fresh water fish farm.

Keywords: Toxicity, haematological, *Cocos nucifera*, water, Catfish *Clarias gariepinus*

1. Introduction
Cocos nucifera Linn. Belonging to the family Arecaceae, is commonly known as Coconut in English. The coconut palm is cultivated in more than 90 tropical countries and it represents an important income source. Nigeria, Indonesia, Philippines and India are the major producers and account for about 75% of World production (FOASTAT 2011) [27]. It is a large palm of about 60 to 90 feet height found across the sea levels of the tropical and subtropical world. It is found throughout Nigeria mainly at the Coastal regions. The coconut is known for its great versatility as observed in the many edible, domestic, commercial and industrial uses of its different parts Harries (1978) [23]. Unlike the endosperms of other plants (e.g., Wheat and Corn), the cellularization process in a coconut fruit does not fill up the entire embryo sac cavity, but instead leaves the cavity solution-filled. This solution is commonly known as coconut water and it is of cytoplasmic origin (Janick and Pall 2008) [39]. Nutrients from coconut water are obtained from the seed apoplasm (surrounding cell wall) and are transported symplasmically (through plasmodesmata, which is the connection between cytoplasms of adjacent cells) into the endosperm, (Patrick 2001) [52].
The aqueous part of the coconut endosperm is termed coconut water. Coconut water contains 94% water. Coconut water has been extensively studied in human medicine since its introduction to the scientific community in the 1940s. In its natural form, it is a refreshing and nutritious beverage which is widely consumed due to its beneficial properties to health, some of which are based on cultural/traditional beliefs (Janick and Pall 2008) [39], Sandhya and

Rajamohan 2008 [61], Asian and Pacific Coconut Community, APCC 1994 [14], Seow and Gwee 1984, George and Sherrington 1984 [32], Campbell-Falck *et al.* 2000 [19, 20], Pummer *et al.* 2001 [53], and Anurag and Rajamohan 2003) [5]. It has certain traditional medicinal uses also. The whole coconut tree may be utilized, but the main products are obtained from the fruit: copra and oil, lauric acid, coconut milk, fiber, flour, coconut water (from immature fruit), The consumption of liquid albumen (or coconut water) of the immature coconut is so important to this country, that resulted in deployment of crops aimed mainly to this application, which does not happen in other producing countries. The increasing demand for natural and healthy foods is one factor that has raised the consumption of this drink that reaches around 350 million liters per year in fresh and industrialized form. Besides highly appreciated for its taste and freshness, it is considered an excellent natural isotonic, so it is also consumed for its nutritional qualities.

Despite consumption of green coconut water is very beneficial, the demand increasing for this product generates a very large amount of waste in places such beaches where the consumption of this drink is common. Although the solid endosperm or green coconut pulp is edible, generally only water is extracted from fresh fruit. It helps in avoiding the spots left after it and also a good way to avoid dehydration. Besides its nutritional role, coconut water also some active ingredients that have growth regulatory properties, e.g., cytokinin-type activity, (George and Sherrington 1984) [32]. Some of the most significant and useful components in coconut water are Cytokinins, which are a class of phytohormones Kende, and Zeevaart (1997) [40]. Other components found in coconut water include sugars, sugar alcohols, lipids, amino acids, nitrogenous compounds, organic acids and enzymes [Arditti (2008) Tulecke, *et al.* 1961; Santoso 1996; USDA 2009] [7, 69, 63, 71] and they play different functional roles in plant and human systems due to their distinct chemical properties. The present study aimed to establish the toxicity, haematological and Histological profile of *Cocos nucifera* water in juvenile Catfish *Clarias gariepinus.*

2. Materials and Methods

Two hundred live and apparently healthy juvenile Catfish *Clarias gariepinus* measuring 11.5 - 14.6 cm total length and 65.6 - 112.4 g weight were identified using taxonomic key of Reed *et al*. (1967). The specimen used for the experiment was collected from Cross River University of Technology fish farm at Calabar, Obubra Campus. The specimen was acclimated for 1 week in the laboratory inside transparent, rectangular glass tanks (75 x 45 x 45 cm) of 121.5 L capacity. The tanks were filled with 50 L unchlorinated well water. The fish was fed to apparent satiation twice daily (0900 and 1600 h) with a commercial pelleted fish diet containing 35% crude protein during the acclimation period. Feeding was discontinued 48 h before the commencement of the experiment to minimize the production of wastes in the test container. The maximum admissible toxicant concentration established for adult tilapia was derived by multiplying a constant 0.01 - 0.1 by 96 h LC50 (Koesomadinata, 2000) [46].

2.1. Water quality analysis

Water quality monitoring was done prior to the experiment, during the experiment and after the experiment. pH was determined using a digital pH meter (Mettle Toledo 320). The electrode was inserted into the bottle containing the water sample after standardization in different buffer, after which the reading was taken. DO_2 was measured using a digital, dissolved oxygen meter (Jenway, 9071) once in a day at 8.00 a.m. While, temperature was measured using a mercury in-glass thermometer, which was placed in the medium inside the test container until reading was taken. The reading was taken at 10.00 a.m. on each day of the experiment.

2.2. Preparation of aqueous extract of Coconut water and acclimation of test fish

240 mature fruits of coconut were purchased from the farmers around Obubra village, in Cross River State, Nigeria. The seed powder was prepared by opening the mature fruit of pawpaw and the fresh seeds extracted and were sun dried. The seeds were ground to a fine powder, using the coffle mill attachment of a Moulinex domestic food blender. The powder was kept in desiccators for later use in stock solutions. A preliminary range finding test was conducted to determine the toxicity level of pawpaw seed powder using standard method (American Public Health Association APHA, 1987).

2.3. Acute toxicity test

The preliminary range finding test was conducted to determine the actual concentration for the test. One control and five tests in triplicates were set up for the experiment. Pawpaw seed powder was introduced randomly, and tested for 24 h, the behavior and mortality of the test fishes in each tank was monitored and recorded every 15 min for the first hour, once every hour for the next 3 h and every 4 h for the rest 24 h period. Eighteen (75 x 45 x 45 cm) glass tanks of 121.5 L capacity each were filled with 50 L aerated dechlorinated well water. Adult *O. niloticus* was batch-weighed with a top-loading mettler balance (Mettler Toledo (K), and distributed randomly in triplicate per treatment. The glass tanks were covered with mosquito net to prevent fish from jumping out; there was no aeration, no water change nor feeding throughout the test. This was done prior to the introduction of the toxicant. The toxicant was introduced at 2, 4, 6, 8 and 10 mg/l with a control of 0 mg/l in triplicate. The test lasted for 24 h.

The definitive test was conducted using concentration of 4.0, 4.2, 4.4, 4.6, 4.8 and 5.0 mg/l of pawpaw seed powder earlier determined for the range finding test. This test comprised one sublethal toxicity test according to the standard method/procedures (American Public Health Association, 1987). Fish mortality was monitored and recorded hourly for the first 4 h, 4 h for the next 24 h, and subsequently every 24 h, for the next 96 h. The inability of fish to respond to external stimuli was used as an index of death. Apart from monitoring and recording fish mortality the fish behaviour such as erratic swimming, air gulping, loss of reflex, discolouration, and molting was monitored. LC_{50}, which is the concentration of pawpaw seed powder, estimated to be lethal to 50% of test organism after exposure time of 96 h, was determined graphically using probit transformation (Herwig, 1979; USEPA, 2000) [38, 78]. Haematological examination of fish followed the method described by Svobodova *et al*. (1991) [70].

The moisture, ash and other extracts like crude fibre, crude fat, and protein were then determined using standard methods (AOAC, 1990) [7]. Nitrogen was determined by the micro-kjedahl method (Pearson, 1976) and the crude protein was taken as N% x 6.25 (constant factor) where N is equal to Nitrogen content per 100g sample. Total carbohydrate was determined using the phenol-sulphuric acid method (Adeyeye

and Faleye, 2004) [4]. The crude fibre was obtained by dry ashing of the sample at 550ºC dissolved in 10% HCl(25ml) and 5% Lanthanum Chloride (2ml) boiled, filtered and made up to standard volume with distilled water. Na and K were determined by the Jenway 1997, Flame photometer method. Phosphorus was determined using the spectronic 20 calorimeter by the phosphovando molybdate method (AOAC, 1990) and (Dubois *et al.,* 1956). Ca and Mg were obtained by EDTA method (Ademoroti, 1996) [3], while the heavy metals were determined using atomic absorption spectrophotometer.

2.4. Heamatological Analysis
Blood (1-2ml) was sampled from groups of fish by inserting a syringe into the vertebral caudal blood vessel. The blood was empty into 5ml heparinized blood bottles treated with Ethyl Diamine Tetracetic Acid (EDTA). Blood samples were centrifuged (1500g for 20 min) to obtain plasma. Plasma samples were stored -20°C until analysis of mineral content. Plasma mineral contents (Na^+, K^+, $^-$) were measured in Lloyt Na/K analyser by ISE method. Dietary pH was measured as described by Tarakci. Dry matter (105°C, overnight), ash (550°C, overnight), crude protein (nitrogenx6.25, Gerhard Kjeldatherm, Königswinter, DE), ether extract (Velp Scientifica 148 Solvent Exractor Milan, IT), crude fiber (Ancom 220, Fiber Analyzer.

2.5. Blood Cell Count
System KX-2IN^{TM} Automated Hematology Analyzer was used in blood cell count, the KX-2IN is an ideal hematology analyzer for a clinical satellite laboratory or research testing. It provide a CBN with 17reportable parameter and 3-part WBC differential,

2.6. Histopathological Analysis of Test Organs
At the end of the experiment one fish per each treatment that is one fish per level of concentration was sampled after 96hours of exposure for histopathological analysis. The fish were killed with a blow on the head using a mallet and dissecting kits the gill, liver and skin were successfully removed. The organs were weighed and submersed in 10% formalin for three days after which tissue was dehydrated in periodic acid Schiff's reagent (PAS) following the methods of Hughes and Perry (1976).

The organ was then embedded in malted wax. The tissues were sectioned into thin section (5.7um) by means of rotatory microtone and were being dehydrated and stain with Harris haematoxylin - eosin (H- and E) stain. Bancroft and cook (1994), using microtone and each section was cleared by placing warm water (38 ^{0}c), Where it was picked with clean slides and oven-dried at 58^{0}c for 30minutes to meet the wax. The slide containing sectional materials/tissue was cleared using xylene and graded level of 50%, 70%, 90%, 95%, and 100% alcohol for two minutes each. The section was stained in haematoxylin -eosin for ten minutes. The stained slide was observed under a light microscope at varying X 100 - 500 magnification, section was examined and photographed using an Olympus BH_2 microscope filled with photographic attachment (Olympus C_{35} AD_4), a camera (Olympus C_{40} AB_{-4}) and an automated light experience unit (Olympus pm (35p).

2.7. Statistical analysis
All results were collated and analysed using computerized, probit and logit analysis (Lichfielf and Wilcoxon, 1949). The median lethal concentration LC_{50} at selected period of exposure, and an associated 95% confidence interval for each replicate toxicity test was subjected to logit and probit analysis (Finney, 1971) using Statistical Package for Social Sciences (SPSS) 17.0 for Windows Vista.

3. Results
3.1. Toxicity
The 96 hour LC_{50} of coconut water to juvenile catfish *(Clarias gariepinus*) is presented in table 1 is 250mg/l and the graphical method of determination of LC_{50} is presented in Figure 1-6 and the concentration of the 100% fish is 750mg/l. The percentage cumulative mortality of coconut water to juvenile catfish *Clarias* gariepinus is presented in table 2 and 3. And fig4 to 9 the values are 0,250,350,450,550,650 and 750mg/l the concentration of the treatment required bringing about 50percent mortality of *Clarias gariepinus* juvenile within 96 hour period. The admissible toxicant concentration of 2.5 - 25mg/l established for juvenile Catfish, was derived by multiplied the 96-hLC_{50} with an application factor of between 0.1 -0.01 according to *Koemsomadinata (1980)* [41]

Table 1: The LC_{50} value for Cocos nucifera to Catfish juveniles

S/n	Time	LC_{50}	MATC
1	20hr	750mg/l	7.5mg/l - 75mg/l
2	24hr	650mg/l	6.5mg/l -65mg/l
3	36hr	550mg/l	5.5mg/l - 55mg/l
4	48hr	450mg/l	4.5mg/l - 45mg/l
5	72hr	350mg/l	3.5mg/l -35mg/l
6	96hr	250mg/l	2.5mg/l - 25mg/l

The acute toxicity of *Cocos nucifera* increases with increase in concentration with time. The total mortality 0f catfish juveniles occurred at a concentration of 750mg/l of coconut water. The maximum toxicant concentration of 2.5mg/l-7.5mg/l established for catfish juveniles was derived by multiplying the 96hours LC_{50}

3.2. The method of determination of LC_{50} using graphical method

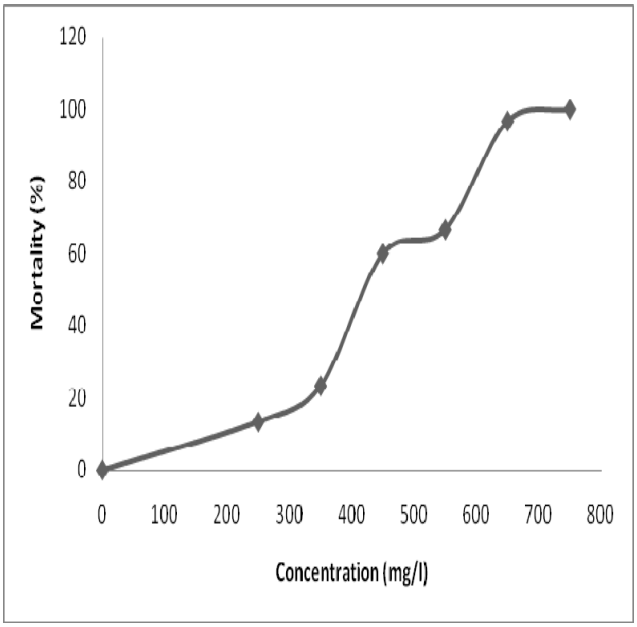


Fig 4: Determination of LC_{50} 0f Coconut water to juvenile catfish (*Clarias gariepinus*) at 20hr

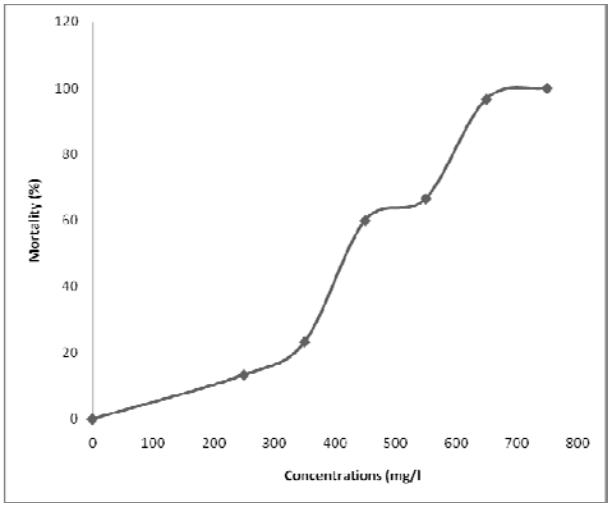


Fig 5: Determination of LC_{50} of coconut water to juvenile catfish (*Clarias gariepinus*) at 24hrs

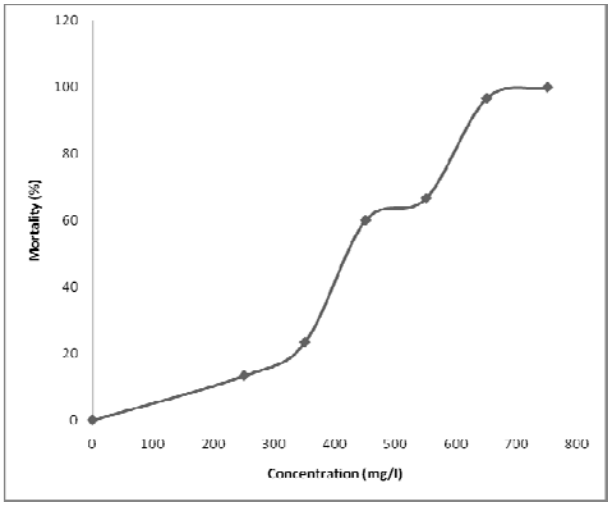


Fig 6: Determination of LC_{50} of juvenile Catfish (*Clarias gariepinus*) to coconut water at 36hrs

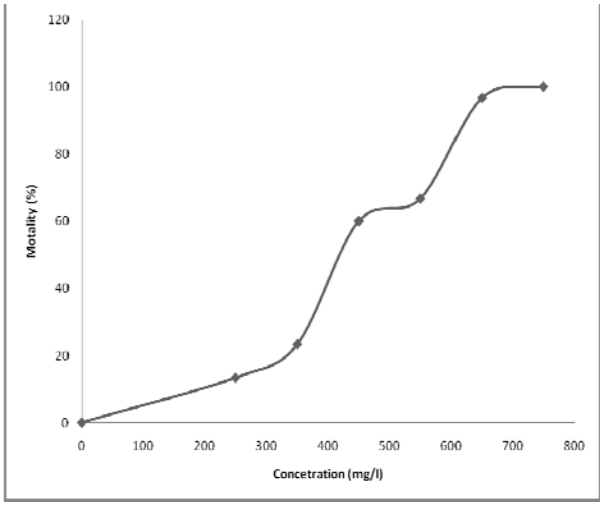


Fig 7: Determination of LC_{50} of Coconut water to Juvenile catfish (*Clarias gariepinus*) at 48hrs

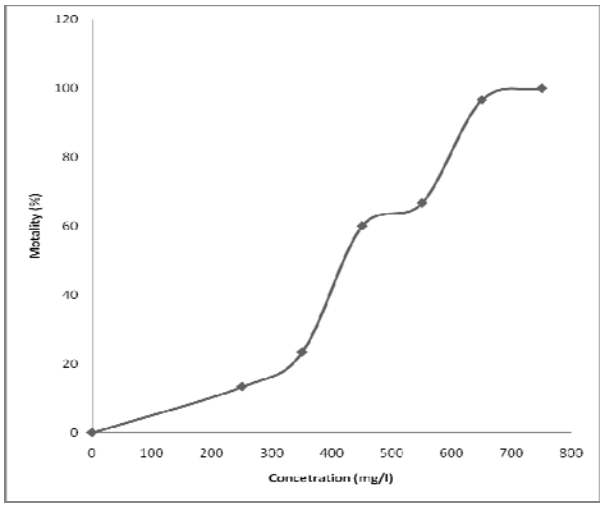


Fig 8: Determination of LC_{50} of Coconut water to Juvenile catfish (*Clarias gariepinus*) at 72hrs

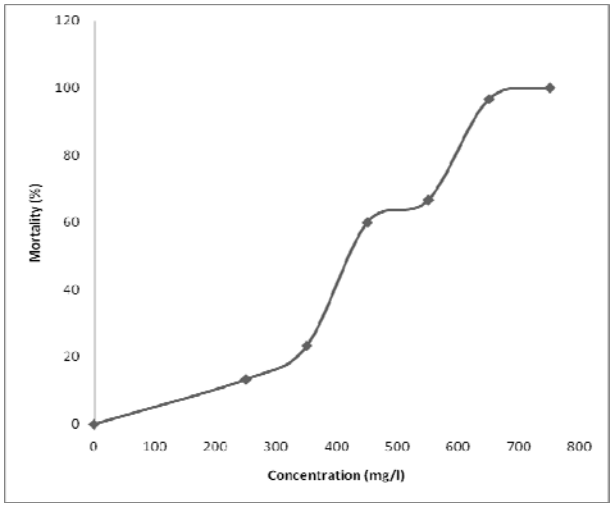


Fig 9: Determination LC_{50} coconut water to juvenile catfish *(Clarias gariepinus)* at 96hrs

3.3. Observation of Range finding Test of Catfish Juvenile *Clarias gariepinus* Exposed to coconut water

The change in the behavior of catfish juveniles observed in the range finding test is summarized in Table 2. During the range finding test, juveniles of *Clarias gariepinus* exposed to coconut water exhibited different behaviors in relation to concentration, such as air gulping, molting erratic swimming, loss of reflex, operculum movement, fin movement, discoloration. The abnormal behavior displayed by the fish exposed to *Cocos nucifera* increased with increasing concentration.

3.4. Observation of definitive Test of Catfish juvenile exposed to Coconut

Erratic swimming was observed in the fish; they also exhibited discoloration, loss of reflex air gulping. The behavioral change observed from the definitive test are summarized in table 3. Discloration was observed to be directly proportional to the increase in the concentration of the coconut water and duration exposure.

Tables 4: Length-Weight Relationship and Condition Factors of Catfish (*Clarias Gariepinus*)

Conc	Weight (Gm)	Standard Lenght	Conc	Weight (Gm)	Standard Lenght
Range Finding Test			**Definitive Test**		
0mg/l	61.10 ± 6.4	18.70 ± 0.4	0mg/l	61.110±6.39	18.89±038
150mg/l	66.50 ± 5.2	20.47 ± 1.9	250mg/l	66.50±5.22	20.47±1.93
350mg/l	66.03 ± 6.8	19.51 ± 0.9	350mg/l	66.04±5.6.78	19.52±0.95
450mg/l	64.03 ± 4.7	19.57 ± 0.7	450mg/l	64.03±4.75	19.57±0.67
600mg/l	57.33 ± 6.5	18.72 ± 0.7	550mg/l	57.34±6.55	18.72±070
750mg/l	61.97 ± 7.0	18.68 ± 1.2	650mg/l	60.80±7.19	18.69±1.16
900mg/l	63.67 ± 6.3	19.42 ± 0.8	750mg/l	63.36±1.40	19.14±1.12

Table 5: Summary of Water Quality Parameter of Coconut Water to Catfish Clarias Gariepinus (Mean±Sd)

Conc.	Temp.(0_c)	Do (M/L)	Ph	Conc.	Temp.(0_c)	Do (M/L)	Ph
	Range Finding				**Definitive**		
$T_{o(0mg/l)}$	25.67±0.58	7.50±0.27	6.64±0.15	T_o	24.67±*11.15*	7.24±*0.47*	7.17±*0.33*
$T_{1(150mg/l)}$	25.34±0.58	7.27±0.38	7.14±0.24	$T_{1(250mg/l)}$	25.34±*0.58*	7.27±*0.15*	7.19±*0.21*
$T_{2(30mg/l)}$	25.34±0.58	7.17±0.11	7.10±0.29	$T_{2(350mg/l)}$	25.10±*0.90*	7.00±*0.27*	7.09±*0.22*
$T_{39450mg/l)}$	25.57±0.58	6.88±0.37	7.45±0.18	$T(450mg/_{l)}$	25.34±*0.58*	6.80±*0.10*	7.27±*0.11*
$T_{4(600mg/l)}$	25.34±0.58	5.77±0.47	6.86±0.23	$T_{4(550mg/l)}$	24.34±*0.58*	6.80±*0.16*	8.35±*0.39*
$T_{5(750mg/l)}$	25.00±0.00	5.80±0.27	6.87±0.20	$T_{5(650mg/l)}$	24.68±*0.58*	7.07±*0.25*	7.14±*0.23*
$T_{6(90mg/l)}$	25.67±0.58	5.07±0.38	7.40±0.20	$T_{6(750mg/l)}$	25.00±*1.00*	7.10±*0.31*	8.27±*0.21*

Table 2: Percentage Cumulative Mortality of *Clarias garepinus* Exposed to Coconut Water (Range Finding Test)

Trt/conc	15mins	30mins	45mins	60mins	2hrs	3hrs	4hrs	6hrs	8hrs	10hrs	16hrs	20hrs	24hrs
0mg/l	0	0	0	0	0	0	0	0	0	0	0	0	0
150mg/l	0	0	0	0	0	0	0	0	0	0	0	0	0
300mg/l	0	0	0	0	0	0	0	0	0	0	0	0	0
450mg/l	O	0	0	3.34	6.67	6.67	13.34	13.34	13.34	16.67	20	20	20
600mg/l	0	0	0	3.34	13.34	16.67	16.67	16.67	16.67	23.34	23.34	33.34	33.34
750mg/l	0	0	3.34	6.67	16.67	16.67	26.67	26.67	33.34	36.67	36.67	43.34	46.67
900mg/l	0	0	3.34	13.34	26.67	30.0	36.67	43.34	50	70	76.67	90	93.34

Table 3: Percentage Cumulative Mortality of *Clarias garepinus* Exposed to Coconut Water (Definitive Test)

Conc.	1hr	2hrs	sss3hrs	4hrs	6hrs	8hrs	12hrs	16hrs	20hrs	24hrs	36hrs	48hrs	72hrs	96hrs
Omg/l	0	0	0	0	0	0	0	0	0	0	0	0	0	0
250mg/l	0	0	0	0	0	0	0	0	3.34	6.67	10	10	13.34	13.34
350mg/l	0	0	3.34	3.34	6.67	10	10	10	13.34	13.34	16.67	16.67	23.34	23.34
450mg/l	0	0	0	3.34	10	23.34	23.34	23.34	26.67	26.67	33.34	40	40	60
550mg/l	0	0	0	0	0	6.67	13.34	20	20	33.34	40	46.67	60	66.67
650mg/l.	0	3.34	13.34	16.67	16.67	20	33.34	33.34	43.34	50	66.67	70	76.67	96.67
750mg/l.	3.34	13.34	30	63.34	30	46.67	50	50	53.34	63.34	76.67	83.34	86.67	100

Table 6: The Summary of effect of coconut water on haematological parameters of juvenile catfish *Clarias gariepinus* (Mean±SD)

conc./mg/l	White blood cell (ul)	Red blood cell (ul)	Haemoglobin (g/dl)	Haematocript (%)	Mean cell volume (fl)	Mean cell haemoglobin (pg)	Platelet (ul)	Lymphocyte (%)	MCH	RCD-WS	RCD-WCV
0	1.69±33.96	6.37±6.87	9.63±2.44	25.27±5.59	1.23±8.24	46.50±1.60	1.98±16709.68	97.700±0.45	37.94±1.50	39.30±2.44	11.10±0.52
250	1.48±25.12	1.61±3.58	8.60±1.21	19.47±3.75	1.18±2.48	52.43±3.57	4.65±12182.09	91.84±4.22	44.60±2.26	59.64±8.44	19.03±0.56
350	1.97±103.87	9.50±9.70	6.60±2.08	10.37±11.55	1.01±11.15	16.44±28.47	5.61±20802.64	31.77±55.02	14.33±24.82	16.70±28.92	51.54±27.78
450	1.60±18.73	1.21±1.05	7.80±1.90	20.06±3.45	1.20±2.53	45.50±4.79	1.80±13568.09	95.70±2.06	37.74±3.75	46.20±15.6	20.50±4.44
550	1.77±13.98	2.09±4.60	11.40±2.36	22.94±4.58	1.10±5.72	56.53±18.67	6.72±41315.98	93.30±5.70	51.60±18.41	24.54±21.39	20.37±9.38
650	1.97±70.35	5.27±7.35	1.97±3.40	6.24±8.87	1.90±69.10	14.37±24.28	6.87±3592.12	31.20±54.04	12.00±20.97	11.00±19.05	3.90±6.75
750	1.14±100.06	1.30±1.12	5.47±4.77	15.03±13.08	1.07±66.78	28.00±22.59	3.60±3377.86	64.84±56.16	24.20±20.97	22.00±19.06	10.2±8.87

Table 7: Summary of effect of coconut water on Biochemical parameters of catfish *Clarias gariepinus* r (Mean ± SD)

Treatment	Moisture (%)	Fibre (%)	Protein (%)	Ether Extract (%)	Ash (%)	Nitrogen Free Extract (%)	Energy (kcal/100g)
0mg/l	73.78±10.73	00.00±00	27.01±0.42	3.76±0.34	0.62±0.54	0.50±0.08	1.44±2.10
250mg/l	64.24±6.37	00.00±00	27.72±1.22	2.82±0.67	0.85±0.47	0.37±0.17	1.41±1.55
350mg/l	69.52±0.74	00.00±00	26.33±1.17	3.13±0.18	0.88±0.09	0.73±0.15	1.36±1.87
450mg/l	69.80±1.39	00.00±00	26.28±0.92	2.1±0.58	0.84±0.14	0.26±0.04	1.35±1.72
550mg/l	68.73±0.65	00.00±00	27.06±0.18	3.22±0.14	0.84±0.44	0.37±0.58	9.33±68.07
650mg/l	68.74±0.58	00.00±00	27.229±1.12	3.09±0.58	0.89±0.09	0.46±0.78	1.34±4.50
750mg/l	68.13±0.38	00.00±00	27.42±0.46	3.17±0.09	0.88±0.12	0.37±0.06	1.39±1.41

Table 8: Summary on plasma electrolyte of Catfish to coconut water

Treatments	Sodium Na (мmol)	Potassium K (мmol)
0mg/l	1.06±4.83	40.54±3.43
250mg/l	73.76±5.49	38.77±5.66
350mg/l	67.27±11.32	36.34±12.62
450mg/l	1.10±74.57	56.40±44.23
550mg/l	55.77±17.57	30.17±3.65
650mg/l	70.24±36.69	22.34±4.17
750mg/l	74.54±26.09	24.14±10.34

Table 9: Biological Monitoring of Catfish Juvenile of *Clarias gariepinus* Exposed to Differents Concentration of Coconut Water (Range finding Test)

Behavior/Exposure Time	12hrs	16hrs	20hrs	24hrs
Concentration ((Mg/l)	O 150 300 450 600 750 900	O 150 300 450 600 750 900	O 150 300 450 600 7509 900	O 150 300 450 600 750 9 900
Air Gulping	- - + + + + +	- + + + + + +	- + + + + + +	- - + + + + +
Erratic Swimming	- - - - - - -	- - - - - - -	- + - - - - -	- + - + - - -
Molting	- - - - - - -	- - - - + + -	- + - + + + +	- - - - - - -
Discoloration	- - - - - - -	- - - + - + +	- + - + + + +	- + - - + + +
Barbell Deformation	- - - - - - -	- - - - - - -	- - - - - - -	- - - - - - -
Tall beat frequency	- - + + - - -	- - - - - - -	- - - - - - -	- + - - - - -
Excessive mucus secretion	- - - - - - -	- - - - - - -	- - - - - - -	- - - - - - -
Operculum Movement	- + + + + + +	- + - + + - +	- + + + - + +	- + + + + + +
Loss of reflex	- - - + - - -	- + - + + - +	- - + - - + +	- - - - + + + -
Fin movement	- + + + - - -	- + + + + + -	- - + - - + +	- + + + - + -

Table 10: Behavioral Monitoring of Catfish Juvenile of *Clarias gariepinus* Exposed to Different Concentration of Coconut Water (Definitive Test)

Behavior/Exposure Time	24hrs							48hrs							72hrs							96hrs						
Concentration ((Mg/l)	0	250	350	450	550	650	750	0	250	350	450	550	650	750	0	250	350	450	550	650	750	0	250	350	450	550	650	750
Air Gulping	-	+	+	+	+	+	+	-	+	-	+	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+
Erratic Swimming	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-
Molting	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	_	-
Discoloration	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	+	-	+	+	-
Barbell Deformation	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-	+	-	-	-	-	+	-	-
Excessive mucus secretion	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
Tall beat frequency	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	+	-
Operculum Movement	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-
Loss of reflex	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	+	-	-	-	+
Fin movement	-	+	+	+	+	+	+	-	-	+	+	+	+	+	-	+	+	+	-	+	-		-	-	-	-	-	-

Table 11: Histological changes in the liver of *Clarias gariepinus* exposed *Coconuut Water*

Concentration (mg/l)	Figures 1-7	Histological changes
Omg/l	1	Normal hepatic tissue, showing normal hepatocytes with no architectural defect
250mg/l	2	Section showing liver tissue with slightly degenerated blood vessel with central vein congested with red blood cell.
350mg/l	3	Section showing normal appearance of the architectural pattern of the tissue but a section of the liver showing fatty changed probably due to hypoxia
450mg/l	4	Section showing ballooning of the hepatocytes and blood vessel congested with blood.
550mg/l	5	Section showing degenerated liver, with thickening of the detachment of the lamella.
650mg/l.	6	Section shows liver tissue with thickened reticular fibres, congested blood vessels with several prominent central vein containing red blood cell.
750mg/l.	7	Section shows degenerated blood vessel with moderate fatty layer.

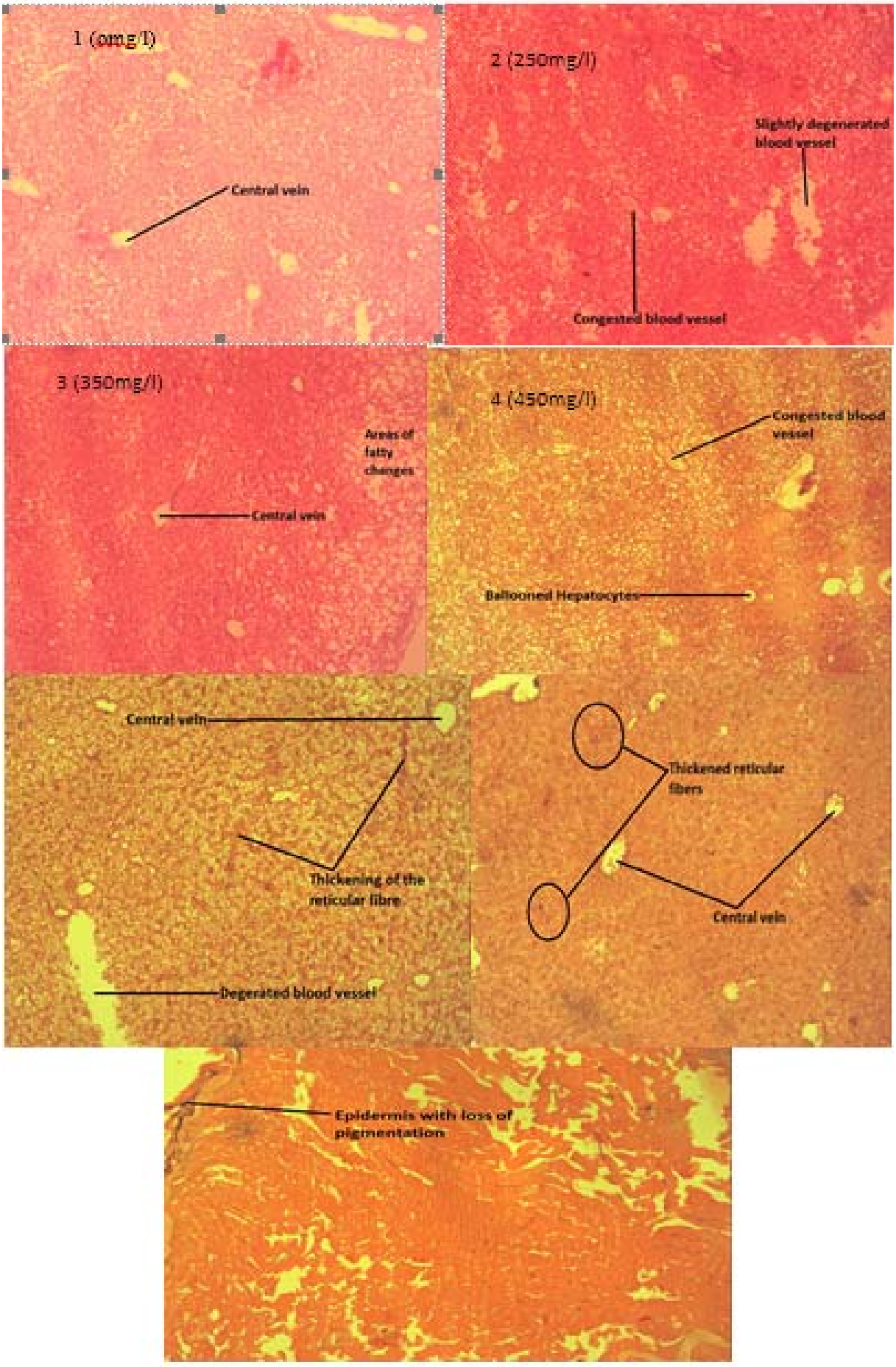

1 (0mg/l)
Central vein
2 (250mg/l)
Slightly degenerated blood vessel
Congested blood vessel
3 (350mg/l)
Areas of fatty changes
Central vein
4 (450mg/l)
Congested blood vessel
Ballooned Hepatocytes
Central vein
Thickening of the reticular fibre
Degerated blood vessel
Thickened reticular fibers
Central vein
Epidermis with loss of pigmentation

Table 12: Histological changes in gill of *Clarias gariepinus* exposed *Coconuut Water*

Concentration (mg/l)	Figures 8-14	Histological changes
Omg/l	8	Normal gill showing the filament, the lamellae and water channel
250mg/l	9	Section of the gill showing water channel filled with red blood cell and the filament of the gills.
350mg/l	10	Section of the gill showing water channel filled with Red Blood Cell (Sign of Trauma)
450mg/l	11	Section of the gill showing haemorrhage in the water channel with detachment of the lamella
550mg/l	12	Section shows haemorrhage in the water with the detachment of the lamella.
650mg/l.	13	Section shows water channel filled with red blood cells, fusion of the lamella and degeneration of the filament.
750mg/l.	14	Section shows degenerated filament, fusion of the lamella and water channel congested with red blood cells.

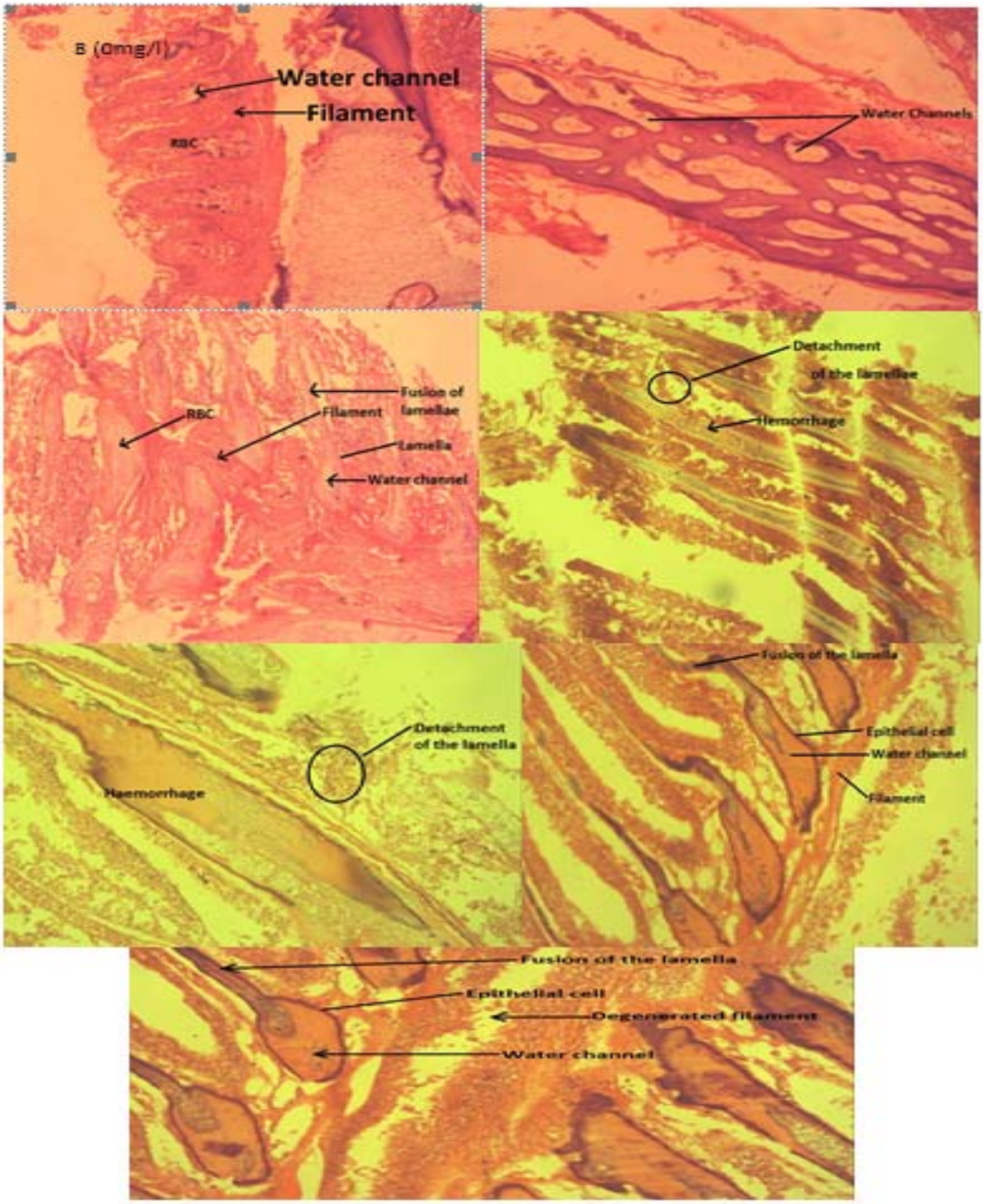
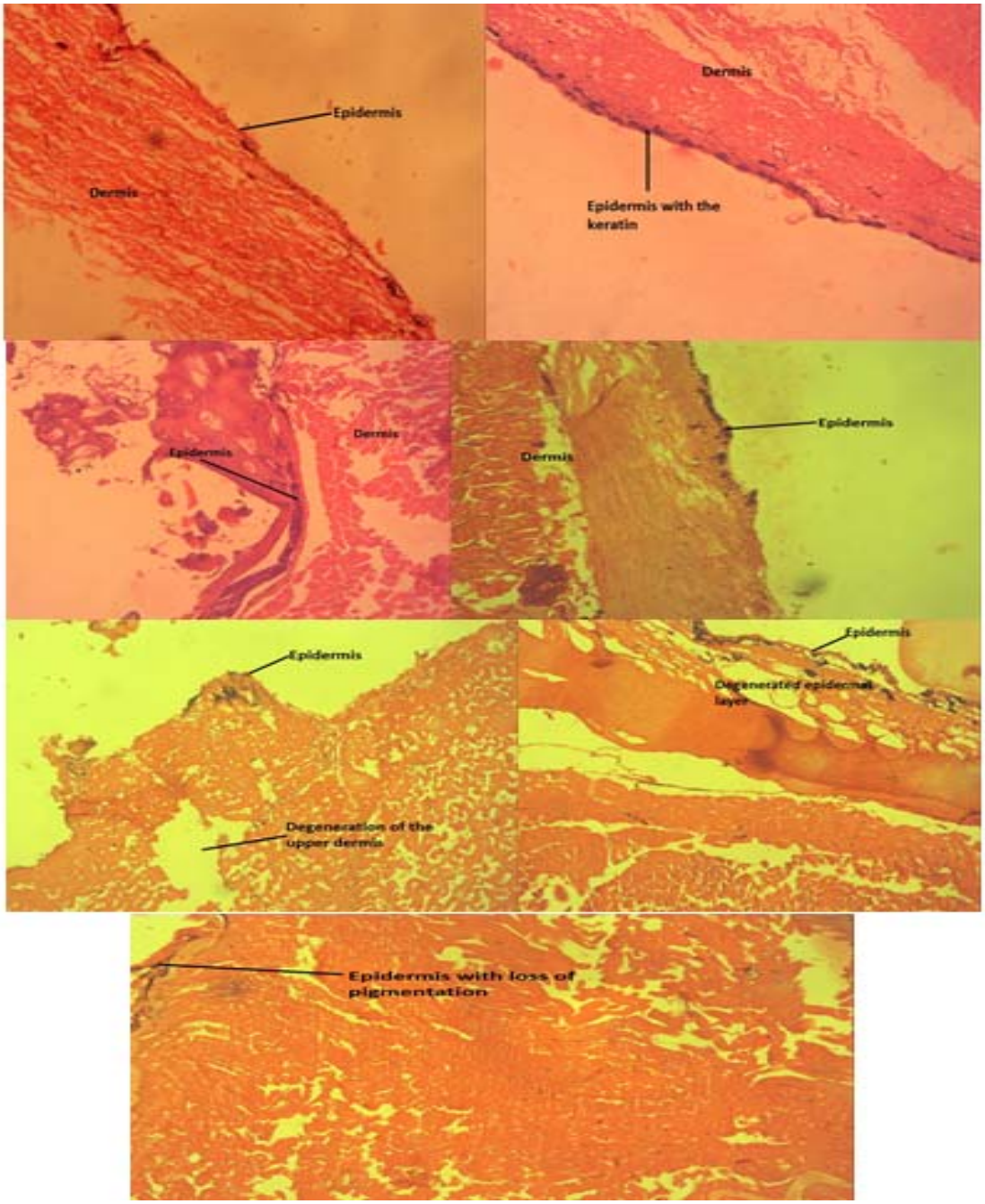


Table 13: Histological changes in skin of *Clarias gariepinus* exposed *Coconuut Water*

Concentration (mg/l)	Figures 15 - 21	Histological changes
Omg/l	15	Normal skin tissue, showing the epidermis and the underlying dermis.
250mg/l	16	Skin tissue showing normal keratin of the skin.
350mg/l	17	Tissue showing normal appearance of the skin with normal keratin without epidermal thickening
450mg/l	18	Normal appearance of the skin with normal keratin appearance.
550mg/l	19	Normal appearance of the skin epidermal layer with no visible thickening of the epidermis and degeneration of the upper dermis.
650mg/l.	20	Section shows keratin that is histologically abnormal, with severe thinning of the granular layer.
750mg/l.	21	Section shows noticeable loss of pigmentation of the keratinlayer.



4. Discussion

The 96hour LC_{50} of *Cocos nucifera* to juvenile African Catfish *Clarias gariepinus* is presented in table 1, and fig 4-9 is 2.5mg/l with 95% confidence interval 47.1576 -100.3957 and the maximum safe concentration range between 2.5-25mg/l to 7.5 -75mg/l, Koesoemadinata (1980) [41], he stated that the safe level of a compound is derived by multiplying the 96 -hLC_{50} with an application factor of 0.1 -0.01 such application factor

are applied to acute toxicity test data to estimate the concentration that is safe for chronic exposure and is closely related to the work of Ayuba and Ofojekwu (2002) [16] who reported the effect of acute toxicity of the root extract of Datura innoxia to African catfish (*Clarias gariepinus*) fingerlings and reported that the 96hourLC_{50} was 204.17mg/l with lower and upper confidence limit of 125.89 and 384.59mg/l. The 96h LC_{50} in the present result is lower than the result reported by Oti (2002) [50] who reported 96hLC_{50} of 50.12mg/l on the toxic effect of cassava mill effluent on the African *Catfish Heteroclarias* hybrid of *Heterbranchus bidorsalis* male and *Clarias gariepinus* female

The result of haematological parameters of *Cocos nucifera* to juvenile African Catfish *Clarias gariepinus* is presented in table 6 which shown increases in red blood count, RCD-WS, RCD-WCV from 39.30 ±2.44, 11.10 ± 0.52, 1.97 ±103.87 and 1.98±16709.68 in control to 59.64 ±8.44, 51.54± 27.78, 1.97 ± 70.35 and 6.87 ±3592.12, in the concentration of 250mg/l, 350mg/l and 650mg/l, agrees with the work of Annune *et al.* (2002) [11] who reported increase in red blood count RCD-WS, RCD-WCV in lower concentration of ringworm plant, *Senna alata* used in poisoning water.

The result of biochemical parameters of *Cocos nucifera* to juvenile African Catfish *Clarias gariepinus* is presented in table 7 shows an increase in Ash and Protein from 0.6± 0.54 and 27.01 ± 0.24 in control to 0.88 ± 0.12 and 27.42 ± 0.46 in the concentration of 750mg/l is close to the work of Orue, and Uner,(1999) [24] who reported increase in liver protein follow in exposure to 2,4 Diamine for 30 days and they was a decrease in moisture, ether extract, nitrogen free extract and energy, from 73.78 ±10. 73, 3.76 ± 0.34, 0.50±0.08 and 1.44 ±2.10 in control to 68.13 ±0.38, 3.17 ± 0.09,0.37 ±0.06 and 1.39 ± 1.41 in higher concentration of 750mg/l and is similar to the work of Bradified and Rees (1978) who reported that toxicants act by disruption of cell membrane permeability replacing the structural or electrochemical important elements in cell which cause functional failure of the organism.

The result of plasma electrolytes *Cocos nucifera* to juvenile African Catfish *Clarias gariepinus* is presented in table 8, which showed increase in sodium from 1.06±4.83 in the control to 74.54±26.09 and the potassium(K) value exhibited similar trend to sodium from 40.54 ±3.43 in 0mg/l to 24. 14 ±10.34 in 750mg/l, the sodium and potassium were significantly($p < 0.05$)lower than the exposed groups than the control recorded in this work is in agreement with the work of Kori-siakpere (2000) [42] who reported the electrolytes response to sub-lethel concentrations of potassium permanganate in African Catfish *Clarias gariepinus* in higher concentration of 750mg/l similar result to the work of Etsler and Edmunds (1906) who observed an increase concentration of sodium, potassium and chlorine in the blood plasma of *Clarias gariepinus* exposed to various concentration of glyphosate herbicide.

The behavioural respons of Clarias gariepinus agreed with the work of Aleem (1988) [10], who observed an increase in erratic swimming and loss of reflex for the test fish as the concentration of tobacco dust were increase and this could probably be due to irritation from increased nicotine content of tobacco

5. Conclusion

This study was conducted to determined effect of coconut water to a cultivable fish species, the African catfish (Clarias gariepinus) juveniles. The 20, 24, 36, 48, 72 and 96LC_{50} of coconut water to juvenile catfish were 750mg/l, 650mg/l, 550mg/l, 450mg/l, 350mg/l, and 250mg/l. While the total mortality occurred at a concentration of 750mg/l within 24hours exposure period respectively. Haematological examination shown an increase in blood cell count (RCD-WS), (RCD-WCV), and Platelet from, 39.30 ±2.44, 11.10 ± 0.52, 1.97 ±103.87 and 1.98±16709.68 in control to 59.64 ±8.44, 51.54± 27.78, 1.97 ± 70.35 and 6.87 ±3592.12, in the concentration of 250mg/l, 350mg/l and 650mg/l, while there was a decrease in Haematocript, MCV, MCH, LYMH, HMB, RBC from,25.27 ±5.59, 1.23 ± 8.24, 46.50±1.60, 97.700 ± 0.45, 37.94 ± 1.50, 9.63 ± 2.41 and 6.37 ±6.87 in control to 15.03 ± 13.08, 1.07 ± 66.78, 28.00 ± 22.59, 64.84 ±56.16, 24.20 ±20.97, 5.47 ± 4.77 and 1.30 ±1.12 in higher concentration of 750mg/l throughout the test in juvenile catfish *Clarias gariepinus*. This work is close to the work of Olalade *et al*., (2010) [46], who reported that *Clarias gariepinus* fingerlings exposed to sub - lethal concentration of *D. innoxia* root extract for 12 weeks caused a significance decrease in RBC, HB, and MCV valves.

The result of biochemical parameters shows a decrease in moisture, ether extract, nitrogen free extract and energy, from 73.78 ±10.73, 3.76 ± 0.34, 0.50 ± 0.08 in the control to 68.13 ±0.38, 3.17 ± 0.09, 0.37 ± 0.06 and 1.39 ±1.41, in higher concentration of 750mg/l, Also there was an increase in Ash and Protein, from 0.6 ± 0.54 and 27.01 ±0.42 in the control to 0.84 ± 0.14 and 26.28 ± 0.92 I the concentration of 350mg/l. In the plasma electrolyte of catfish juvenile to coconut water, there was an increase in sodium from 1.06± 4.83 in the control to 74.54 ±26.09 in the concentration of 750mg/l, while in potassium; there was a decrease from 40.54 ± 3.43 in the control to 24.14 ±10.34 in higher concentration of 750mg/l. Behavioral responses of African catfish Clarias gariepinus to coconut water include: air gulping, operculum movement, loss of reflex, discoloration, erratic swimming.

The result of histopathological responses of skin, gill and liver of the test organisms to aqueous extract of coconut water showed different level of degeneration of cells, hypertrophy of gill arch, disarrangement of hepatic cell, necrosis, vacuolation, sign of trauma the gill, detachment of lamella, vessels congested with blood vessels, liver hypoxia, degeneration of the upper dermis and severe thinning of the underlying granular layer. Damages became severs with increase in concentration of aqueous extract and time exposure. There are no significant changes in the water quality during and after the experiment. The result of the tests provided the baseline information and established safe limits of using coconut water in fresh water fish farm. The fish finally settled at the bottom motionless with slow operculum movement. The results of physio-chemical parameters obtained before the test, during the test and after the test showed that the death of fish is not as a result of poor water quality but coconut because in the control the fish do not undergo any behavioral sign or death. The result of the tests provided the baseline information and established safe limits of using coconut water in fresh water fish farm.

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