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Piscicidal effects of methanolic castor seed extract on catfish juveniles

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Abstract

Traditional plants have been found useful for various purposes by the fish artisanals, but their toxicity on the fish is hardly considered. This study assessed the toxicity of castor (*Ricinus communis*) seed extract on catfish (*Clarias gariepinus*) juveniles in a static renewable bioassay. The study involved 500 fish samples collected from Motherhood Fish Farms, Nigeria. A stock solution of the methanol seed extracts, diluted in ratio 1:1 with water, was used for each treatment of the bioassay media concentrations in triplicates of 0 (control), 5, 6, 7 and 8 g L^{-1}; following high mortality in 10 g L^{-1} determined in a finding range test. The LC_{50} and LC_{100} were calculated using probit method. The results showed that the irregular behavioural patterns and mortality rate of the test fish increased with increasing concentrations of the extracts at the intervals of 12 to 96 hr exposure. The serum results revealed that total protein values were significantly ($p<0.05$) different from the control. Levels of albumin increased in the fish blood serum, globulin fluctuated, while creatine, potassium and alkaline phosphatase decreased as the extracts' concentrations increased over the control treatments. The gill structures showed deformation and liver showed cirrhosis across the concentrations but no recognisable changes on the control fish. There were adverse effects from extract concentrations 5 g L^{-1} and above. Therefore, indiscriminate use of castor seed on water bodies threatens the existence of catfish due to its piscicidal effects.

Key words: *Clarias gariepinus*, fish toxicology, histopathology, phytochemicals, *Ricinus communis*

Introduction

Pharmacological studies have ascertained uses of plants for various purposes without recognising their toxicity potential (Ekor, 2014). Plant are recognised for having enormous natural components that make them toxic to humans (Awasthy *et al.,* 2000). Although bioactive constituents in plants are supposed not to cause health problems (Vaghasiya *et al.,* 2011), some compounds are toxic unless used appropriately (Kirtikar and Basu, 1975). Plants have shown toxicity to zooplanktons (Kreutzweiser *et al.,* 2004), shrimps (Goktepe *et al.,* 2004) and commercial fish species (Obomanu *et al.,* 2007). A number of compounds such as resin, nicotine, saponins, tannins, alkenylphenols, di- and tri-terpenoids and diosgenin are present in plants including the castor and can cause injury to fish. These are explored by man to catch fish (Obomanu and Fekanurhobo, 2005; Tiwari *et al.,* 2008). Though studies on medicinal plants usage have been carried out, their toxicity remains unexplored (Wojcikowski *et al.,* 2004). Plant toxicity is evaluated through physical behaviours, blood parameters assessment and histopathological observation of animals (Sasidharan *et al.,* 2008).

An example of plant with piscicidal effect is castor (*Ricinus cummunis*) which belongs to the spurge family (Euphorbiaceae) containing number of plants that are native to the tropics. It belongs to a monotypic genus *Ricinus* and sub-family *Ricininae.* Castor seed contains some compounds that are toxic to fish, for instance ricin and ricinine. Castor seeds, on the other hand, provide some nutritional benefit for the pond once the toxicity has gone away (after 13-14 days) and have been observed to deliver more nitrogen to the pond than Mahua oilcake. Ricine's toxicity is mostly due to its suppression of protein synthesis, although other mechanisms such as apoptotic pathways, direct cell membrane damage, changes in membrane shape and function, and inflammatory mediators have also been documented. This study assessed the toxicity of castor seed (*Ricinus communis*) seed extracts on catfish (*Clarias gariepinus*) juveniles in a renewable bioassay.

Methodology

Study site

The experiment was carried out at the Fish Hatchery Centre of the Department of Aquaculture and Fishery Management, Federal University of Agriculture, Abeokuta, (FUNAAB), Ogun State, Nigeria. The site is located at 7°13'23.6"N, 3°25'25.3"E. The experiment was conducted using 15 rectangular plastic tanks of diameter 50 cm x 25 cm x 30 cm.

Castor seed samples

Castor seed samples were collected from the Federal University of Agriculture, Abeokuta (FUNAAB) Arboretum (Teaching Farm) and nearby village (Kofesu, Alabata and Abeokuta), with the assistance from the Department of Forestry and Wildlife Management, FUNAAB. The samples were kept in aluminum foil, and then processed into powder according to the method of Cal *et al.* (2005). The powder form allowed easy release of castor oil for extraction (Akpan *et al.,* 2006). Methanol (1000 mL) was used as solvent for extraction, following the method of Tounou *et al.* (2011a). The solution produced was then heated in a water bath to evaporate the solute (methanol) and effectively produce the treatment (methanolic castor seed extract) to be used.

Experimental fish culture

Catfish (*Clarias gariepinus*) in the form of juveniles was used in this study because of its easy reaction to change in the surroundings unlike the easily adapted nature its adult (Odiete, 1999). A total number of 500 catfish juveniles (~ 14 g) was procured from Motherhood Fish Farms, Obantoko, Abeokuta, Ogun State, Nigeria. The fish were transported in a plastic water container (50 L capacity) to the hatchery unit of FUNAAB; and transferred to the tanks to acclimatise for a period of two weeks (14 days), where they were fed with 2 mm extruded feed. The feed remnants and fecal materials were removed by using a siphoning tube (a hose).

Catfish (*Clarias gariepinus*) is a wild fish that has been domesticated with the ability to adapt to diverse environment with poor water quality due to its accessory breathing organs, along with its high fecundity rate, wide habitat preferences, environmental tolerance as well as the ability to feed on a wide range of prey (Omeru and Solomon, 2016). Such characteristics make the fish the best species (Omeru and Solomon, 2016) for toxicological experiment. Information on the toxicity castor seed extracts will help to address the health and environmental risks usage may pose for fishing communities.

Experimental design

Treatments included 5, 10, 15 and 20 g of the blended castor seeds in 1000 mL methanol as the extracts. The treatments were laid out in completely randomized, adopting a renewal bioassay (where the media are periodically changed) for the 96 hr test. The treatments were replicated three times and the study was repeated four times.

Preliminary test

From the acclimatisation tanks, 15 fish (~14 ± 0.6 g weight and 7 ± 0.8 cm standard length) were stocked per experimental tank. They were deprived of feed for 24 hr prior to this preliminary (finding rage) test, which was used to determine the suitable range of concentrations for the static bioassay. Any observed fecal materials and dead filth were removed from the tank to prevent pollution. A stock solution of the methanol seed extracts, diluted in ratio 1:1 with water, was used to prepare the four different treatments (5, 10, 15 and 20 g L^{-1}). The control (without the test extract) made up the fifth experimental tank. High mortality rates were recorded except, below 10 g L^{-1}, thus less concentrations were determined for this study.

Experimental test

Five treatments of 0 (control), 5, 6, 7 and 8 g L^{-1} were prepared in triplicates for the 96 hr experiment. The choice of these five treatments followed the above range finding test results, where there were massive deaths of fish above the treatment of 10 g L^{-1}. The five treatments were renewed in the various experimental tanks every 24 hr after changing the water to maintain potency of the extract. The water quality parameters, observed behaviours of the fish, and mortality were monitored every 12 hourly. The 24 hr LC_{50} (sub-lethal) and LC_{100} (acute-lethal) were then determined using probit analysis.

Water quality analysis

Water quality parameters (temperature, pH, conductivity and dissolved oxygen) were monitored *in situ* at 12 hr intervals, using the digital Hanna instrument (model: 211, microprocessor pH meter). The dissolved oxygen level of the water was taken with the Griffin Oxygen Meter (Model: 40), the ammonia level of the water was determined using acid method for nitrogen (APHA, 1998).

Blood sampling and serum analysis

At the end of the 96 hr experiment, five fish per tank (n = 25) were randomly selected and anaesthetised with 100 mg L^{-1} clove oil. Blood was collected from the caudal arch of the fish using a 25-gauge needle and 1 ml syringe. Subsequently, the blood was transferred into heparinized tube for basic haematological analysis. The haemoglobin, red blood cell count, white blood cell count, and differential leucocyte count were determined according to the standard methods of Lewis *et al.* (2006). The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated as described in Haney *et al.* (1992) using the following formulae *viz.*:

MCV = haematocrit (%PCV) 10) /RBC (x109 L^{-1})
MCH = (haemoglobin (g dL^{-1}) 10) /RBC (x1012 L^{-1})

MCHC = (haemoglobin (g dL^{-1}) 100) /haematocrit (%PCV)

Blood was collected from another five fish in each tank (n = 25) without the use of an anticoagulant. Samples were subsequently centrifuged at 3000 xg for 10 min at 4 °C and the collected serum was stored in 20 °C for biochemical analysis. The serum samples were used to determine the total protein (Doumas, 1994), albumin (Reinhold, 1988), and globulin was calculated by subtracting the albumin value from the total protein value of the same sample (Coles, 1998). The activity of serum aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were determined using commercial kits (Randox Laboratories Limited, Crumlin, United Kingdom).

Histopathological assessment

The five fish per tank (n=25) used for the blood sampling and serum analysis were also used for the gill and liver histological examination. The samples were fixed in 10% neutral buffered formalin, and processed in graded ethanol and xylene. The processed tissues were embedded in paraffin wax, sectioned (5 µm thickness) and stained with periodic acid-Schiff or haematoxylin and eosin. The processed histological slides were imaged using a light microscope (BX53, Olympus Life Science, Tokyo, Japan) and morphological measurements were carried out using ImageJ (version 1.51, National Institute of Health, Bethesda, Maryland, USA).

Results

Phytochemical analysis

The analysis of castor seed samples using methanol as solvent revealed six biologically active phytochemicals, with terpenes and tannins present in minute quantities; while alkaloids, glycosides, flavonoids and saponins were present in substantial quantities (Table 1).

Water quality analysis

The analytical quality of water in this study is represented by data in Table 1. All the parameters considered, except conductivity, and dissolved ammonia were not significantly ($p > 0.05$) different across the extract treatments.

Results of the preliminary study on acute- (LC_{50}) and sublethal (LC_{100}) concentration

The results of acute lethal (LC_{50}) and sublethal tests (LC_{100}) according to the probit graph, showed that 12.9 g L^{-1} concentration would influence 50 % mortality; while 22.4 g L^{-1} concentration had a total (100 %) mortality of the catfishes (Fig. 1). The

Table 1. Castor seed phytochemicals and water quality parameters for the 96 hour test

Phyto-constituents						
	Alkaloids	Glycosides	Flavonoids	Terpenes	Saponins	Tannins
Quantitative (%)	0.8815	0.0745	0.0022	-	1.2315	-
Qualitative	+	+	+	+	+	+
Extract treatment						
Conc. (g L^{-1})	0	5	6	7	8	p<0.05)
Temperature (°C)	26±0.05	25±0.11	25±0.15	26±0.13	27±0.11	0.056
pH	6.5±0.03	7.0±0.06	7.2±0.05	7.9±0.04	8.7±0.05	0.061
Ammonia (mg L^{-1})	0.0	0.0048±	0.0053	0.0077	0.0082	0.035
Dissolved oxygen (mg L^{-1})	6.6±1.32	6.3±1.50	5.7±1.13	5.6±1.15	5.1±1.20	0.058
Conductivity (µS cm^{-1})	131±5.1	146±7.2	158±8.5	165±9.3	178±10	0.044
Preliminary test results						
R. communis seed extracts and the corresponding probits						
Conc. (g L^{-1})	20	15	10	5	0	
Log Conc. of dose	1.301	1.176	1.000	0.699	-	
Mortality (%)	80.0	45.0	23.5	0.0	0.0	

Key: - Absent/ minute quantity, + Present in trace amounts

graph that puts probability of mortality against the logarithm of concentration of extract was used. This informed that lower concentrations than 10 g L^{-1} from the preliminary (finding range) test should subsequently be chosen for the 96 hr toxicity test. The result obtained from this study showed that methanol extract of castor seed had toxic effects on the catfish juveniles and the effect of their toxicity increased with concentrations of the bioactive extract substances in the setup.

Fish behaviour

There were no significant changes in the test juvenile catfish behavior in the lower (5 and 6 g L^{-1}) concentrations of the castor methanol extract extracts during the 96 hr acute toxicity studies (Table 2). However, when placed in the bioassay tanks, the juvenile fish showed uncoordinated movement and aggressiveness with timeless attempts to jump out of the tanks for the treatments at 7 and 8 g L^{-1}. These behaviours continued for few minutes (approximately 1 hr); after which the movement became normal and calm. Exposure to the extracts showed different behaviours (Table 2)

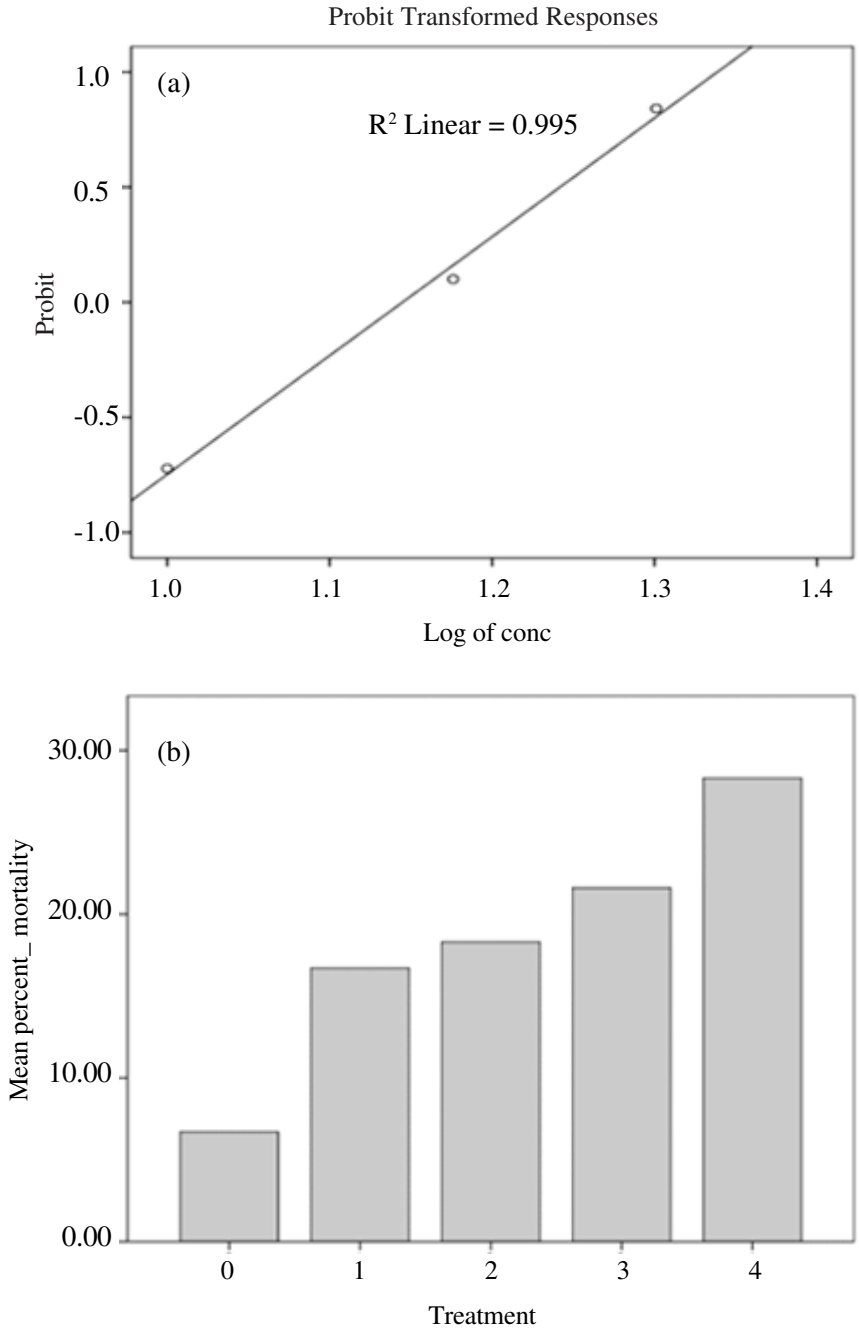

Figure 1. (a) Log (dose of concentration) versus probits, and (b) percentage mortality-treatment relationship for 96 h test (where 0, 5, 10, 15 and 20 g L^{-1}).

Table 2. Behaviour and blood serum readings of *C. gariepinus* juveniles and methanolic *R. communis* extract (g L^{-1})

Behaviour of the fish samples in different concentrations					
Treatments	Control	5.00	6.00	7.00	8.00
Loss of reflex	Absent (-)	Absent (-)	Low (+)	Mild (++)	High (+++)
Hyperventilation	Absent (-)	Absent (-)	Absent (-)	Mild (++)	High (+++)
Erratic swimming	Absent (-)	Absent (-)	Low (+)	Mild (++)	High (+++)
Suffocation	Absent (-)	Absent (-)	Low (+)	Mild (++)	High (+++)
Spiral movement	Absent (-)	Absent (-)	Low (+)	Mild (++)	High (+++)
Blood serum parameters of the fish samples in different concentrations					
Treatments	Control	5.00	6.00	7.00	8.00
Total protein	4.2 ± 0.00^{a}	4.37 ± 0.06^{a}	4.57 ± 0.06^{b}	4.57 ± 0.12^{b}	5.07 ± 0.06^{c}
Globulin	1.63 ± 0.06^{a}	1.37 ± 0.06^{b}	1.50 ± 0.10^{ab}	1.90 ± 0.00^{c}	1.87 ± 0.06^{c}
Albumin	2.67 ± 0.12^{a}	2.87 ± 0.06^{b}	3.17 ± 0.12^{c}	2.60 ± 0.00^{a}	3.27 ± 0.06^{c}
Creatine	1.20 ± 0.00^{a}	1.07 ± 0.12^{b}	0.93 ± 0.06^{c}	0.73 ± 0.06^{d}	0.53 ± 0.15^{e}
Astaxhantine	72.67 ± 0.58^{a}	72.00 ± 3.00^{a}	103.33 ± 2.08^{d}	67.68 ± 2.31^{b}	101.67 ± 1.73^{c}
Alt	26.33 ± 0.58^{a}	25.67 ± 0.58^{a}	48.33 ± 2.89^{c}	26.33 ± 2.31^{a}	34.00 ± 1.73^{b}
K	4.20 ± 0.00^{a}	4.17 ± 0.06^{a}	5.10 ± 0.10^{d}	3.83 ± 0.21^{b}	4.57 ± 0.25^{c}
Alp	26.33 ± 1.15^{a}	31.67 ± 0.58^{b}	25.67 ± 1.15^{a}	38.68 ± 0.58^{c}	41.00 ± 1.00^{d}

Value with different superscript across the rows were significantly different at $P< 0.05$. Alt= Alanine transaminase (IU L^1), K= condition factor, Alp = Alkaline phosphatase (IU L^1)

that included increased physical activity, incessant gulping of air, irrational swimming patterns, rapid uncoordinated movements and loss of balance, whitening of the whole body, unusual inactiveness and motionless with slow opercula movements prior to death.

Blood serum parameters and Histopathological changes

There were noticeable changes in the serum biochemistry (Table 2) and haematological parameters (Table 3) across concentrations (0 (control), 5, 6, 7 and 8 g L^{-1}) of the methanolic castor seed extracts compared to control fish.

The exposition of catfish to the varied methanolic castor seed extract treatments showed several gills histological alteration (Plate 1). There was loss of epithelial cell architecture with increased vacuolation in the cell in 5 g L^{-1} (2 on Plate 1), partial loss of the gill cellular architecture in 6 g L^{-1} (3 on Plate 1). There was partial loss in the gill cellular lamellae, epithelial cellular architecture in 7 g L^{-1} (4 on Plate 1). Meanwhile, there was complete loss of the gill lamellae and the epithelial cellular arrangement in

Table 3. Haematological parameters of *Clarias garipienus* juvenile after 96 h

Blood parameters: control (0)		5 g L^{-1}	6 g L^{-1}	7 g L^{-1}	8 g L^{-1}
PCV	18.00±2.00^{ab}	17.002.00^{a}	22.332.08^{ab}	19.333.21^{ab}	23.004.58^{b}
WBC	6.331.26^{ab}	10.331.76^{c}	9.731.62^{c}	6.001.00^{a}	8.871.60^{bc}
RBC	1.300.75^{a}	1.170.55^{a}	2.000.50^{a}	1.970.25^{a}	2.130.42^{a}
Haemoglobin	6.330.61^{ab}	5.670.85^{a}	7.330.76^{bc}	6.670.90^{ab}	8.500.75^{c}
Heterophil (%)	22.672.31^{b}	34.675.51^{c}	15.331.53^{a}	29.333.06^{c}	31.003.61^{c}
Lymphocyte (%)	66.676.51^{bc}	52.005.29^{a}	73.336.11^{c}	58.338.62^{ab}	56.674.16^{ab}
Monocyte (%)	0.670.58^{ab}	0.670.58^{ab}	0.330.58^{a}	1.670.58^{bc}	2.330.58^{c}
Eosinophil (%)	0.670.58^{a}	0.330.58^{a}	0.330.58^{a}	0.670.58^{a}	1.000.00^{a}
Basophil (%)	1.330.58^{a}	0.670.58^{a}	1.670.58^{a}	0.670.58^{a}	1.330.58^{a}

Values with the same superscript across the rows were not significantly different at $p > 0.05$. PCV, WBC and RBC represent packed cell volume

8 g L^{-1} (5 on Plate 1) compared with the gill epithelium of normal gill of the control experimental fishes (1 on Plate 1). Further histopathological observations revealed normal liver histology in 5 g L^{-1} methanolic caster seed extract (7 on Plate 2), the liver might have detoxified the possible influences of the determined phyto-toxins thereby leaving no change in the histo architecture of the liver. There was an indication of hepatic necrosis and fatty infiltration on the liver in 6 g L^{-1} concentration (8 on Plate 2). There were increases in hepatic necrosis and fatty infiltration with accompanied disruption of the blood vessel at 7 g L^{-1} extract concentration (9 on Plate 2). On the liver of the fishes in 8 g L^{-1} concentration, liver revealed massive necrosis of the hepatocytes, replacement of most of the hepatocytes with fat as the liver tended towards cirrhosis (10 on Plate 2). However, the liver of the control fish showed no change (6 on Plate 2).

Discussion

Phytochemical analysis

The phytochemical screening of castor seed extract revealed presence of active phytochemicals in varying quantities. The qualitative analysis of castor seed extract showed that glycosides, flavanoids, terpenes, alkaloids, saponins, and tannins were present in trace amount. These findings are similar to what was reported from a bioassay evaluation by Thombre *et al.* (2013) who found phyto-constituents that included alkaloids, tannins, steroids, triterpenoids, and saponin from a methanol extraction.

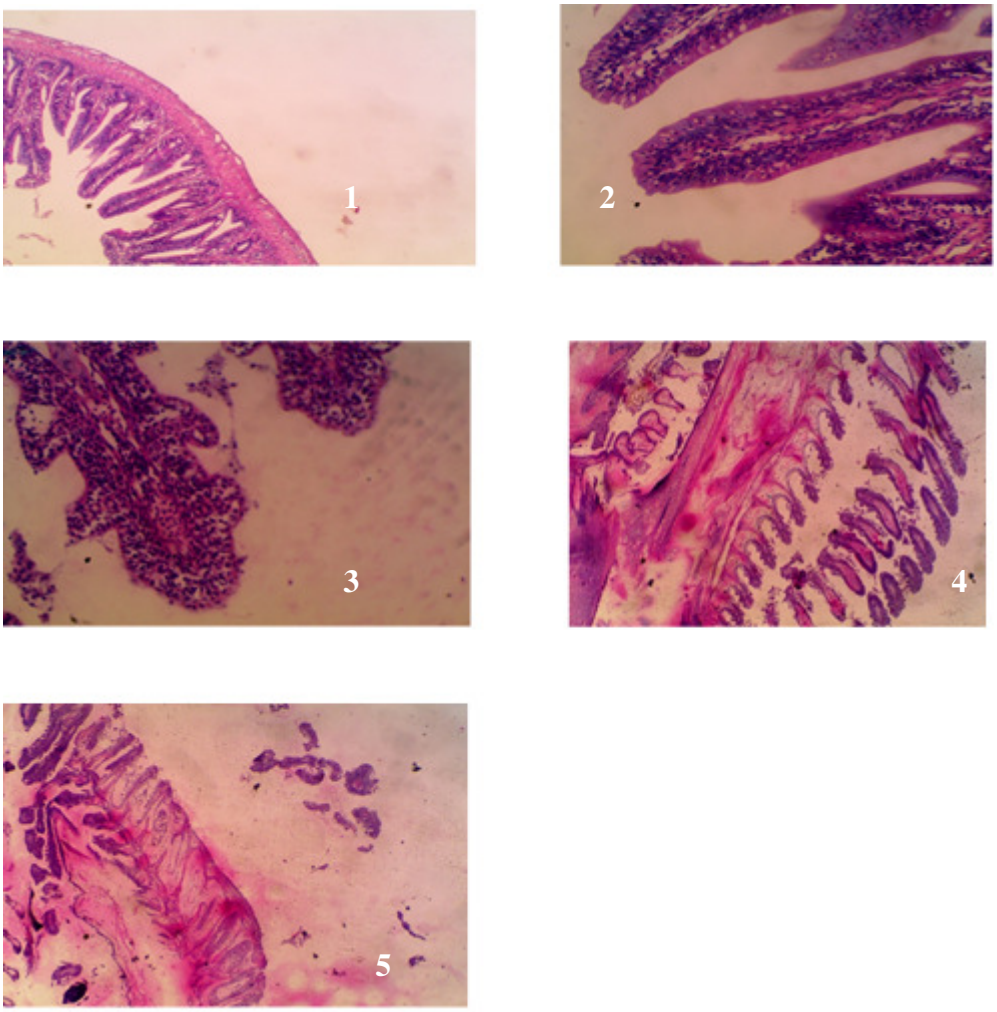

Plate 1. Responses of the experimental (2-5) and control (1) test fish gill samples to the methanolic castor seed extract treatments X 400 E.

Acute- (LC_{50}) and sublethal (LC_{100}) concentration

The determined bioactive phytochemicals in the castor seed have provided the benefits for traditional wide range treatment of chronic and infectious diseases (Duraipandiyan *et al.,* 2006). They are used for fish catch as a result of their piscicidal effects, though without careful evaluation of their extract effects. The toxicity nature of most plants made the World Health Organization declare that prerequisite steps (which include infusion, bioassay, isolation, characterization and toxicological evaluation) should be taken before exploring biologically active chemicals of plants for any benefit (Sasidharan *et al.,* 2011). However, the quantity and types of the phytochemicals to be determined or extracted depend on the solvents and methods of extraction (Olujimi *et al.,* 2017; Idowu *et al.* (2019, 2021).

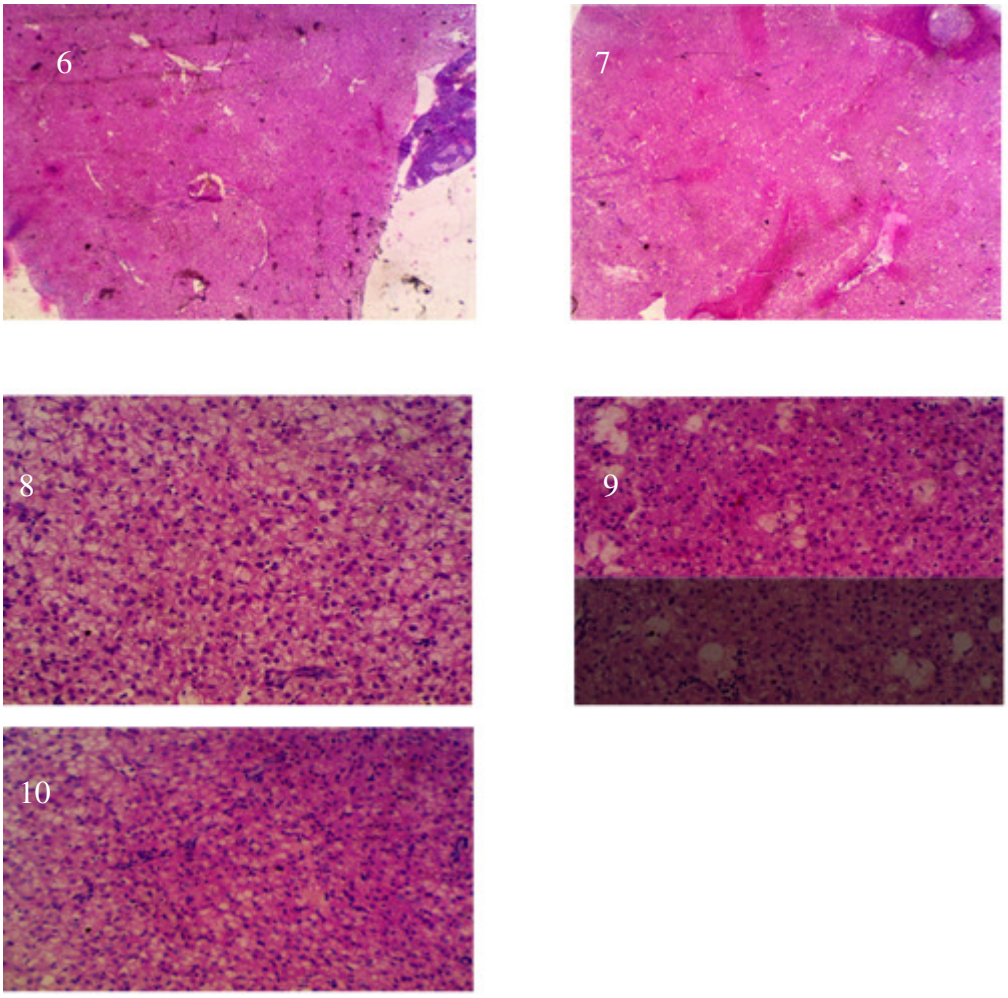

Plate 2. Responses of the experimental (7-10) and control (6) liver samples to the methanolic castor seed extract treatments X 400 E.

Water quality analysis

The values of temperature, pH and dissolved oxygen determined in this study were consistent with values of Federal Environmental Protection Agency (FEPA, 1991), which put the optimal ranges for fish culture at 20-30 $^{\circ}$C for temperature, 6-9 for pH and above 5 mg L^{-1} for dissolved oxygen. Temperature is an important physical factor which affects biological reactions in aquatic ecosystems, and influences other physicochemical characteristics (Soundarapandian *et al.,* 2009). Fish experiences slow growth at pH < 6.5, its ability to maintain salt balance is affected at lower pH and production might stop (Loyd, 1992), while at nearly pH 4 or at least pH 11 most species die (Lawson 1995). As pH increased, so did the values of ammonia in the water and probably its toxicity to the fish as reported by the USEPA (2014). At high

pH, ammonia toxicity prevails more (Boyd, 1990). The ammonia concentration varied but remained at an acceptable level according to FAO (2012) discovery that ammonia level in water above 0.02 mg L^{-1} was considered toxic to fish. Acute toxicity of ammonia to fish had been extensively studied and the results were largely consistent, indicating that ammonia levels can cause death in fish at values higher than the USEPA standard (Brinkman, 2009). Values of the dissolved oxygen became lower but conductivity became higher as the concentration of methanolic extracts of castor seed increased. Dissolved oxygen impairs fish growth if it is below 75 % saturation for long, so it should be maintained at minimum of 95 % saturation for optimal growth (Romaire, 1985).

Fish behaviour and mortality

The difference in the level of toxicity of the extracts could be linked to the concentrations of the phytochemicals in the plant under study (Idowu *et al.* 2019, 2021). This further proved that the lethal effects increased as the concentration of extract increased. Therefore, acute toxicity study revealed that the presence of methanol extract of castor seed after 96 hours of exposure on catfish led to inactivity and death at concentrations above 10 g L^{-1}. Lethal oral toxicity determination is the leading step in the screening and evaluation of toxic potentials of phytoconstituents (Akhila *et al.,* 2007). The LC_{50} toxicity studies of herbal medicines are essential to ascertain the safety, determine dose levels which could be used subsequently, helps in the investigation of the therapeutic index of drugs and xenobiotics (Rang *et al.,* 2001).

Toxicity effects are analysed using fish parameters like behaviour, hematological and histological analysis (Ahmad *et al.,* 2013). Idowu *et al.* (2019) reported unrest movement of catfish exposed to water extracted phyto-constituents. The abnormal behaviour of the fish may be due to suffocation leading to forceful respiration, and could be compared favourably with the observation of Omoniyi *et al.* (2013) at different concentrations of phyto-constituents on catfish juvenile.

An important rule for toxicity tests is that the beneficial treatments are valid if they cause less than 10% mortality (Odiete, 1999). The highest (8 mg L^{-1}) castor seed methanolic extract had low percentage mortality of 6.7 % in this study. The range of normal values of importantly active biochemical parameters remained largely undefined for different species and conditions (Gayatri and Prafulla, 2014). This study indicated the maximum dosage of 8 mg L^{-1}, beyond which toxicological effects could be imposed on the test catfish.

Blood serum and parameters

The values of total protein levels in the test fish blood serum gradually increased along with the treatments (Table 2). Igwebuike *et al.* (2008) attributed alteration of the total protein in fish to the condition of the environment (which are the added treatments in this study) and nutritional status. An increase in total protein concentration of the test fish blood serum can be caused by structural liver alterations thereby reducing alanine aminotransferase (ALT) activity and impairing fluid balance control (Coz-Rakovac *et al.,* 2005). The values of globulin decreased in 5 and 6 g L^{-1} treatments but then increased steadily in the higher extract concentrations (Table 2). All the values remained in the range of normal albumin (2.5 to 4.0 g dL^{-1}) as reported by Olfert *et al.* (1980). Activities of liver enzymes exhibited a significant ($p < 0.05$) increase in the blood of fish in the higher levels (7 and 8 g L^{-1}) of methanolic castor seed extract. Both ALT and ALP (alkaline phosphatase) are non-plasma specific enzymes which may give specific information about possible dysfunctional organ: heart, kidney, gills and muscles (Cassilas *et al.,* 1983). There was a noticeable increase in ALP value and the instability of ALT values may have been due to the liver impairment. Increase in ALT and ALP activities in the serum as shown in the study may indicate damage in the cells of the spleen, liver, kidney and other important tissues and organs as reported by Rao (2006).

The lack of significant differences in eosinophil and basophil showed that the methanolic castor seed extract treatments had no major effects on them. The drop in haemoglobin shown in the 5 g L^{-1} treatment might signify anemia (Ojezele and Agunbiade, 2013). This was corroborated in similar fluctuations of an integral part of the Pearson's complete blood count (packed cell volume, PCV) (Table 3), which is the proportion of the blood volume occupied by the red blood cell (RBC) (Purves *et al.,* 2004). There was no significant ($p > 0.05$) difference in the RBC index across the treatments which corroborated the result of Odeyemi *et al.* (2009) who observed that applied extracts neither had effect on erythropoiesis, morphology nor induced RBC osmotic fragility.

The white blood cell (WBC) of the test fish increased rapidly from 6.33 ± 1.26 (in the control treatment) to 10.33 ± 1.76 (in the 5 g L^{-1} treatment) before declining (Table 3). Observations indicated immune response of the test fish to the treatments, because the first line of cellular defense that responds to inflammation, infectious agents or tissue injury is WBC (Tousson *et al.,* 2011b). There was a decrease in WBC count (from 5 to 7 g L^{-1} treatments) to depict a decrease in the leukocytes production (i.e. leukopenia), which is the condition of inability of the body to fight infections influenced by the extracts toxicity (Kasthuri and Ramesh, 2018). From the report of Ezedinma and Oti (2001), nutritive values of food have positive influence

on the fish haematological parameters, and the analysis of blood parameters reports any alterations and evaluate the relative risk effects on the hematopoietic system of the fish under study (Jothy *et al.,* 2011). Haematological parameters in this study served as an index to assess toxicity of the methanolic castor seed extracts.

Histopathological changes

Histopathology is a standard method to evaluate treatment-related pathological changes in tissues and organs (Ajani, 2006). Dosing of the extracts in increasing amounts helps to evaluate the plant toxicity limits (Parra *et al.,* 2001). Observed alteration in the gill structure as the treatment concentrations increase is one of the major indications of the toxicity effects on fish organs (Carol, 1995; Eaton and Klaassen, 1996). The various observations in the current study might be attributed to the individual and combined effects of the different chemicals detected in the methanolic castor seed extracts.

These constituents are liable to cause more damages to the fish tissues/ organs (gill and liver) as the one of primary areas affected by the metabolic reactions (Dybing *et al.,* 2002). The gill and liver alterations implicated large profile of secondary metabolites that were responsible for the toxicity activity (Newman *et al.,* 2003) in castor extract. The liver is the main target organ of lethal toxicity that is exposed to toxic constituents absorbed from the intestines and metabolised into other compounds which may be hepatotoxic (Rhiouania *et al.,* 2008). The organ regenerates from damage and its functions may not be compromised early by a toxicant (Salawu *et al.,* 2009). The toxicity observed in this study is the evidence that castor seed contains several bioactive constituents that had the potential to cause adverse toxic effects (Bent and Ko, 2004). The adverse effects of such constituents manifested in the gill and liver after 96 h, similar to findings of Yamthe *et al.* (2015).

Conclusion

Alkaloids, glycosides, flavonoids and saponins were the phytochemicals quantified in castor seed extracts. The extract exhibited acute lethal toxicity with mortality at treatment higher than 10 g L^{-1} with LC_{50} and LC_{100} at 12.9 and 22.4 g L^{-1} treatments, respectively. Though the treatments of 5-8 g L^{-1} caused fish mortality that was less than 10%; there were significant adverse effects on blood and histological parameters for concern.

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