Pertanika J. Trop. Agric. Sc. 42 (3): 1081 - 1096 (2019)

PERTANIKA JOURNALS

TROPICAL AGRICULTURAL SCIENCE

Journal homepage: http://www.pertanika.upm.edu.my/

Lethal Doses and Histopathological Changes in Liver and Kidney of Healthy *Clarias gariepinus* Sub-adult Exposed to Red *Allium cepa* Linn. Bulb

Oghenebrorhie Mavis Oghenochuko[1,2]*, Johnny Olufemi Olukunle[3], Olubukola Tolulope Adenubi[3], Olusola Lawrence Ajayi[3], Fakilahyel Musa Mshelbwala[3], Ikililu Abdulraheem[2], James Taiwo Apantaku[3], Olabosede Vivian Anuoluwapo Takeet[3], Tunde Isaac Omoniyi[2] and Godfrey Nnamdi Ezeri[2]

[1]*Animal Science Programme, Department of Agriculture, Landmark University, P. M. B. 1001, Omu-Aran, Kwara State, Nigeria*

[2]*College of Environmental Resources Management, Federal University of Agriculture, Abeokuta, P. M. B. 2240, Ogun State, Nigeria*

[3]*College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, P. M. B. 2240, Ogun State, Nigeria*

ABSTRACT

Adverse effects of *Allium cepa* bulb has been well overlooked because it is natural and assumed safe. This study investigated the lethal doses and histopathological changes in liver and kidney of healthy *Clarias gariepinus* sub-adult exposed to red *Allium cepa* bulb at varying concentrations (200, 150, 100, 50 and 25 g/kg) of *A. cepa* via diet and bath (5, 3, 1.5, 0.7 and 0.4 g/L) for two weeks. Specimens were sacrificed, liver and kidneys collected, processed and examined for histopathological changes. Proximate analysis, qualitative and quantitative phytochemistry was determined using standard methods. Brine Shrimp lethality assay (BSLA), LD_{50} and LC_{50} of the onion on experimental fish were investigated. Saponins, tannins, phenols, flavonoids and alkaloids were present. LC_{50} of the onion extract was between 0. 51 mg and 0.64 mg in the BSLA while mean LD_{50} and LC_{50} for dietary and bath treatments were 447.1 g/kg and 12.2 g/L. Liver histopathology showed vacuolar degeneration of the hepatocytes and congestion of central vein, while necrosis of the epithelial cells and

ARTICLE INFO

Article history:
Received: 03 April 2019
Accepted: 24 July 2019
Published: 19 August 2019

E-mail addresses:
oghenochuko.oghenebrorhie@lmu.edu.ng (Oghenebrorhie Mavis Oghenochuko)
drfaks@yahoo.com (Johnny Olufemi Olukunle)
bukiadenubi@gmail.com (Olubukola Tolulope Adenubi)
alabsola@yahoo.com (Olusola Lawrence Ajayi)
mshelbwalafakilahyel@yahoo.com (Fakilahyel Musa Mshelbwala)
a.ikililu@yahoo.com (Ikililu Abdulraheem)
taiwoapantaku@gmail.com (James Taiwo Apantaku)
vivikeet@yahoo.co.uk (Olabosede Vivian Anuoluwapo Takeet)
itomoniyi@yahoo.com (Tunde Isaac Omoniyi)
godfreyezeri@gmail.com (Godfrey Nnamdi Ezeri)
* Corresponding author

ISSN: 1511-3701
e-ISSN: 2231-8542

haemosiderosis were observed in the kidney at high concentrations. *Allium cepa* is safe in fish when administered in lower dose either through diet or bath exposure but was toxic at high dose.

Keywords: *Allium cepa*, *Clarias gariepinus*, histopathology, phytochemistry, toxicity

INTRODUCTION

The use of medicinal plants for human and animal therapy and production performance had been on the rise for decades in which herbal medicine is said to be popular among 70% of the population (Batta, 2012). This is due amongst other reasons to the resistance problems associated with the use of conventional drugs (Nebedum et al., 2009). *Allium cepa* Linn. (onion) is a commonly grown and is second most consumed vegetable in the world (Kuete, 2017). Aside its nutritive values, it has been used for folklore medicine for the treatment of different infections and diseases ranging from trauma, convulsion, hay fever, pneumonia and much more (Patil & Patil, 2007).

Allium cepa had been well documented to have a wide range of therapeutic potentials (Augusti, 1996; Griffiths et al*.,* 2002). It has been reported that variable concentrations of the *A. cepa* bulb administered to fish either via diet and bath improved the growth performance, feed utilization, body composition and survival of different fish species (Obaroh et al., 2018; Saleh et al., 2015). It has also been said to have antimicrobial (Amrevuawho et al., 2016; Bello et al., 2012) and immunostimulatory activities (Saleh et al. 2015). Other studies using other animal models and *in vitro* have also confirmed the antioxidant (Ashwini et al., 2013), antihepatotoxic (Obioha et al., 2009), antilipidemic (Ugwu & Olam, 2011) and anticancer (Nicastro et al., 2015) effects of the onion bulb. However, despite reports that administration of high dose resulted to mortality in experimental animals (Borelli et al., 2009; El-Sayed et al., 2015; Salami et al., 2012), there is dearth of information on the safe dose and pathological effects of *A. cepa* on the internal organs of fish.

Fisheries and aquaculture are important means of livelihood to many farmers and have contributed to the economy of many countries (Martini & Lindberg, 2013). In Nigeria, agriculture contributed 26.15% to GDP in the fourth quarter of 2018 (National Bureau of Statistics [NBS], 2019) to which the fisheries subsector contributes 3-4% (Food and Agricultural Organization [FAO], 2019). *Clarias gariepinus* Burchell is the most cultured fish species in the study area because of its hardiness, adaptability and acceptability. However, in a bit to increase fish production, intensification of the aquaculture sector has led to increased issues of disease outbreak and huge economic loss in this subsector (Opiyo et al., 2018). Hence this study determined the concentration of the *A. ceepa* bulb that can be recommended for use as feed additive and bath treatment to enhance the performance of *C. gariepinus*.

MATERIALS AND METHODS

Experimental Fish

Five hundred sub-adult *C. gariepinus* of average weight 421±2.41g were obtained from a reputable fish farm within Ogun State, Nigeria. Handling during transport was well managed to reduce stress and transportation of fish was in 120 L bowls to experimental site. Fish were distributed into 50 pieces of 80 L bowls at 10 fish/bowl for 14 days for acclimatization. Fish were fed 3 mm commercial diets "Coppens" (Alltech Coppens, Netherlands) twice daily within this period (Table 1). Water quality parameters of temperature (28-32^{o}C), dissolved oxygen (4-6.5 mg/L) and pH (6.5-9) were managed at optimal range by daily change of water and monitored using HANNA multi parameter (Model HI98194) water test kit. Health status of experimental fish was evaluated before commencement of the study by physical observation of the fish activities such as swimming pattern, response to feed, fish skin condition according to the method of Johansen et al. (2006). No clinical sign of disease was observed.

Table 1
Manufacturers' proximate composition of 3 mm Coppens feed

Major components	% composition
Crude protein	45
Crude fat	12
Crude fiber	1.5
Ash	9.5
Moisture content	8.3

Collection, Preparation and Extraction of Plant Bioactive Compounds

Fresh onion bulbs (red variety) were purchased from the local onion market in Abeokuta, peeled, washed using clean water, drained properly and blended using manual blender to obtain a coarse blend. The blended onion (1000 grams) was macerated in different polar solvents: n-hexane, acetone and methanol at a ratio of 1 kg: 1.3 L of solvent and placed in an electronic flask shaker (SM/DR-10, Singifield Medicals, England) for 15 hours. Extraction was by cold maceration method, sieved using Whatman paper (125 cm pore size) and concentration was by the use of a rotary evaporator (Mode: HEI-VAP SILVER, Heidolph Instruments GmbH & Co. KG, Germany) at 40^{o}C to obtain the crude extract. Crude extracts from each solvent were weighed and refrigerated at 4^{o}C for a two day period before commencing of study. Identification and authentication of plant were done at the Department of Forestry and Wildlife Management Herbarium, Federal University of Agriculture, Abeokuta (FUNAAB) and voucher specimen/ID number UAHA: 018/0001 given.

Phytochemical Screening

The phytochemical composition of the onion bulb was determined using standard methods outlined by Harborne and described by Santosh et al. (2013).

Fish Diet Preparation

Pearson square method of fish feed formulation was adopted in the formulation

of six experimental diets containing 40% crude protein as described by Adegbesan et al. (2017). The fresh *A. cepa* bulb meal was incorporated into each of the diet at 0 (control), 2.5, 5, 10, 15 and 20%.

Proximate Analysis of *Allium cepa* and Experimental Diets

The proximate compositions of whole *A. cepa* bulb and experimental diets were carried out as follows: for crude fibre, moisture and fat contents, the method described by the Association of Analytical Chemist (AOAC) (2005) was employed using n-hexane as solvent. The Macro Kjeldahl method of Kirk et al. (1991) was adopted for crude protein and total ash contents determination while percentage carbohydrate content was by differentiation.

Experimental Design for Sub Chronic Toxicity Studies

For sub chronic toxicity studies, complete randomized design was adopted. Experimental fish were grouped into A, B and C. Group A served as dietary treatment group with onion inclusion levels 20, 15, 10, 5 and 2.5% in experimental diets. Group B fish on the other hand were exposed to bath treatment with varying concentrations of 5, 3, 1.5, 0.7 and 0.4 g/L of the onion bulb. Group C was not exposed to any form of onion and served as control. Fish were replicated thrice per treatment with 15 fish/ replicate. Static renewal bioassay system was adopted in this study to maintain a constant onion concentration. Pilot study for the bath treatment was conducted prior to commencing of study. In the pilot study, the onion bulb slurry was introduced into the fish experiment at 20, 15, 10 and 5 g/L. This was done to ascertain the range of concentrations that will not kill the experimental fish immediately. Mortality was monitored and recorded.

Experimental protocols adopted in this study for test fish were according to the internationally accepted standard for the laboratory animal usage. They were approved by Ethics Committee on the Laboratory Animal Use of the College of Veterinary Medicine, FUNAAB.

***In vitro* Assessment of Toxicity of the Onion Bulb**

Brine Shrimp Lethality Assay (BLSA). Using the assay system, 5 Petri dishes were prepared. Each of which contained 20 ml of seawater (35 ppt) well filtered. Two-fold dilution was set up to get different concentrations (2, 1, 0.5, 0.25, and 0.125 mg/ml) of the plant extracts.

For hatchability assay, hatching success of *Artemia salina* cysts in the various petri dishes containing different concentrations of plant extracts and positive control was evaluated using the method of Manilal et al. (2009). Ten (10) individuals of *A. salina* cysts were introduced into each petri dish containing 20 ml of filtered seawater. Petri dishes were covered partly and placed in the incubator at 28°C for 24- 72 hours under steady illumination. After every 24 hours, free nauplii in each petri dish were counted. Hatchability percentage was calculated by comparing the hatched nauplii in individual

petri dish to the total number of cysts stocked (Carballo et al., 2003). Minimum Inhibitory Concentration (MIC) was then determined.

Method of Data Analysis. Complete randomized design was adopted for this assay with 2 replicate incubations per treatment. Mortality data (comprising of *A. salina* that hatched out and died and unhatched cyst) obtained from the different concentrations extracts and control experiments were used to plot dose/response. Their respective LC_{50} values were then determined (Syahmi et al., 2010). LC_{50} values were determined using the probit dose-response curve (Finney, 1952).

In vivo Assessment of Toxicity of the Onion Bulb in *Clarias gariepinus*

Determination of Lethal Dose of *Allium cepa* Slurry on Experimental Fish. The LD_{50} was determined by log-dose/probit regression live method (Finney, 1952). Experimental fish were exposed to 200, 150, 100, 50 and 25 g/kg diets and 5, 3, 1.5, 0.7 and 0.4 g/L of onion bulb via prolonged bath. Experimental fish were observed for 14 days and then mortality was noted for further calculation. Graph empirical probit and log dose was then plotted and LD_{50} calculated using regression analysis

Histopathology

Three fish each were selected from the treated groups and the control. The livers and kidneys from each of these fish were preserved in buffered formalin at 10% concentration. The tissues were trimmed and different concentrations of alcohol (50, 70 80, 90, 100%) was used for dehydration, tissues were then cleared using xylene and paraffin wax used for embedding. Section of 5µm were cut and stained with haematoxylin and eosin according to the method described by Pulvertaft (1950). Sections were then examined under the light microscope.

Data Analysis

All obtained data from the study were expressed in mean ± standard deviation (mean ± SD). One-way analysis of variance (ANOVA) was used to test for the means. Means were then separated using Duncan Multiple Range Test (Duncan, 1955). Probability levels < 0.05 were significant. The LD_{50} and LC_{50} were analyzed for using Probit linear regression Finney (1952).

RESULTS AND DISCUSSION

Proximate Analysis of the Onion Bulb

Proximate analysis of the onion bulb showed high moisture content (83.28%) followed by percentage crude protein (8.48%) and carbohydrate (5.94%) (Table 2). Most of the phytochemicals present in the bulb such as proteins and carbohydrates have good nutritional values. For instance Bhattacharjee et al. (2013) in their study posited that the *A. cepa* plant stored up their carbohydrate as fructans which explained the prebiotics activity of the bulb in the gut of fish thereby conferring on it, its growth promoting activity. Available evidences also suggest that fructooligosaccharide and inulin

Table 2
Proximate analysis of whole Allium cepa *bulb*

Moisture content (%)	Fat content (%)	Ash content (%)	Crude Fiber (%)	Crude Protein (%)	Carbohydrate (%)
83.28 ± 0.01	0.14 ± 0.00	0.5 ± 0.01	1.68 ± 0.01	8.48 ± 0.01	5.94 ± 0.05

present in the onion bulb, acts as prebiotic and influence different physiological activities of fish, such as blood and serum parameters, general performance of the fish including nutrient utilization and body composition, lipids breakdown and blood cholesterol levels (Yarahmadi et al., 2016).

Proximate Analysis and Percentage Composition of the Experimental Diets

Table 3 revealed the mean values and percentage gross composition of the diets used for both experimental and control fish. Crude protein content in all the experimental diets 1,2,3,4,5 and control diets were 40.3±0.06, 40.9±0.02, 40.8±0.11, 40.6±0.10, 40.2±0.02 and 40.0±0.27 respectively. Highest carbohydrate percentage was obtained in diet 1 (40.68±0.67) and least in diet 2 (34.48±0.11).

Phytochemical Analysis of *Allium cepa*

Phytochemical analysis of *A. cepa* extract showed various chemical compounds in

Table 3
Proximate analysis and Percentage composition (%) of the experimental diets

Parameters	1	2	3	4	5	6
Moisture content (%)	4.66±0.06	3.28±0.03	3.94±0.04	3.58±0.088	3.71±0.021	4.25±0.023
Fat (%)	2.50±0.03	6.13±.05	5.19±.06	6.01±.05	5.33±0.01	4.51±0.04
Ash (%)	9.80±0.03	12.20±0.02	11.38±0.02	12.14±0.06	11.88±0.02	10.89±0.02
Crude fiber (%)	2.06±0.07	3.01±0.02	2.46±0.07	2.81±0.06	2.66±0.03	2.18±0.1
Crude Protein (%)	40.3±0.06	40.9±0.02	40.8±0.11	40.6±0.10	40.2±0.02	40.0±0.27
Carbohydrate (%)	40.68±0.67	34.48±0.11	36.23±0.31	34.86±0.19	36.22±0.22	38.17±0.07
Fish meal	23	23	23	23	23	23
Soybean meal	45	45	45	45	45	45
Maize	30	30	30	30	30	30
Fish premix	0.5	0.5	0.5	0.5	0.5	0.5
Dicalciumphosphate	1	1	1	1	1	1
Toxin binder	0.1	0.1	0.1	0.1	0.1	0.1
Salt	0.2	0.2	0.2	0.2	0.2	0.2
Lysine	0.1	0.1	0.1	0.1	0.1	0.1
Methionine	0.1	0.1	0.1	0.1	0.1	0.1
Onion	20	15	10	5	2.5	0

Key: 1 = experimental diet with *A. cepa* inclusion of 200g/kg, 2 = experimental diet with *A. cepa* inclusion of 150g/kg, 3 = experimental diet with *A. cepa* inclusion of 100g/kg, 4 = experimental diet with *A. cepa* inclusion of 50g/kg, 5 = experimental diet with *A. cepa* inclusion of 25g/kg, 6 = control diet with 0g/kg of *A. cepa* inclusion

different composition in which combined anthraquinones had the highest mean value (1.52 ±0.04) while steroids had the least (0.016 ± 0.00) (Table 4). Saponins, flavonoids, alkaloids, phenol, steroids, tannins and cardiac glycosides present in the onion bulb in this study corroborated the study by Ogbonna et al. (2016). Although, Gazuwa et al. (2013) reported the presence of alkaloid, flavonoid, cardiac glycoside and steroid, they however observed that tannins, saponins and total phenols were absent in the fresh onion bulb which was not in agreement with the present study. The difference may be due to cultivar, environment/geographical location, maturity stage, storage time and agronomic conditions (Abayomi & Terry, 2009).

Table 4
Phytochemistry of the onion bulb

S/N	PARAMETERS	QUANTITATIVE %	QUALITATIVE
1	Alkaloid	0.74 ± 0.01	+
2	Flavonoid	0.45 ± 0.01	+
3	Tannin	0.96 ± 0.01	++
4	Saponin	0.27 ± 0.01	+
5	Glycosides	0.55 ± 0.00	+
6	Total phenol	0.97 ± 0.01	++
7	Steroid	0.016 ± 0.00	+
8	Free anthraquinone	0.30 ± 0.00	+
9	Combined anthraquinone	1.52 ±0.04	++

Note. + = presence minimal, ++ = highly present

Brine Shrimp Assay

Hatching success of *A. salina* in the different solvent extracts is represented in Table 5. Hatchability percentage was observed to significantly increase ($P > 0.05$) with length of incubation. With highest hatchability 75% recorded for methanolic extract and least 65% in n-hexane extract. The minimum inhibitory concentration (MIC) was lowest in acetone extract. The mean lethal concentration of all the extracts showed concentrations less than 1mg/ml. Finney probit dose-response graph revealed 'b' values of 2.428, 2.288 and 2.348, 'a' values of 6.229, 6.453 and 6,451 and 'r' calculated was 0.95, 0.88 and 0.97 respectively for methanol, acetone and n-hexane extracts of the onion bulb (Table 5). *Allium salina* cysts hatchability percentage was higher in the methanol extract. However, this was not the result with the LC_{50} value obtained for the methanol extract which indicated higher bioactivity. This finding disagreed with the reports by Ohikhena et al. (2016). They documented that hatchability percentage in the acetone extract of *Phragmanthera capitata* was higher than in the methanol extract which could be attributed to the differences in the test plant species.

Solvent-dependent MIC that was obtained in the BSLA could be attributed to the polarity of the solvents of extraction

Table 5
Dose-response, Percentage hatchability, MIC and LC_{50} of Brine shrimp exposed to Allium cepa *using probit*

Extracts	Dose mg/ml	Total nauplii	Live nauplii	Hatchability (%)	MIC (mg/ml)	Log dose	Mortality	Mortality %	Probit	LC_{50} (mg/ml)	R value
FOBM	2	20	0	0	2	0.3010	20	100	7.44	0.5113	0.9476,
	1	20	4	20		0	16	80	5.84		
	0.5	20	9	45		-0.3010	11	55	5.13		
	0.25	20	12	60		-0.6021	8	40	4.75		
	0.125	20	15	75		-0.9031	5	25	4.33		
FOBA	2	20	1	5		0.3010	19	95	6.64	0.6350	0.8775
	1	20	0	0	1	0	20	100	7.44		
	0.5	20	7	35		-0.3010	13	65	5.39		
	0.25	20	11	55		-0.6021	9	45	4.87		
	0.125	20	14	70		-0.9031	6	30	4.48		
FOBH	2	20	0	0	2	0.3010	20	100	7.44	0.6180	0.9742
	1	20	2	10		0	18	90	6.28		
	0.5	20	6	30		-0.3010	14	70	5.52		
	0.25	20	11	55		-0.6021	9	45	4.87		
	0.125	20	13	65		-0.9031	7	35	4.61		

Key: FOBM – Fresh onion bulb methanol extract, FOBA – Fresh onion bulb acetone extract, FOBH – Fresh onion bulb n-hexane extract

resulting in varying extraction capability. Various studies have shown that solvent polarity determines the phyto compound extracted from a plant (Illoki-Assanga et al., 2015; Widyawati et al., 2014). The result obtained for BSLA of various extracts of the onion bulb suggests that it has minimal toxicity according to the interpretation given by Bastos et al. (2009). Hence, these extracts may be considered safe for use as feed additive but care should however be taken as toxicity could be conferred with high concentration or long duration of exposure. The mild toxicity effect of the onion bulb extract is an indication that the bulb of onion could be used as an alternative in treatment and management of disease conditions, since BSLA is used to indicate presence and level of bioactivity of a plant (Ohikhena et al., 2016).

Clinical Signs of *Allium cepa* at High Concentrations

No clinical signs were observed in sample fish after 14 days of exposure to the onion bulb slurry through fish diets ad bath.

Gross Lesions observed in *Clarias gariepinus* at Postmortem

Paleness of gills, eyes were opaque at 5 mg/ml concentrations of bath treatment. Skin lesions were also observed in sample fish especially at 200g/kg diet treatments. Mortality was highest in 200g/kg diet (Table 6).

Lethal Concentration of *Allium cepa* on Experimental Fish

The minimum lethal dose and concentration of *A. cepa* in *C. gariepinus* were 441.7g/kg for those given as dietary inclusion and 12.2g/L for those used as bath (Table 6). The LC_{50} for experimental fish was low in fish exposed via bath. Finney probit dose-response graph revealed 'b' values of 4.86 and 4.34, 'a' values of -7.71 and +1.62, 'r' calculated was 0.81 and 0.91 respectively for feed and bath exposure to the onion prophylaxis. The 'r' value showed a strong positive relationship between concentration and mortality in both routes of administration.

The lethal dose and concentration calculated for the onion bulb on *C. gariepinus* implied that lower dosage of the plant is considered safe for fish. The mortality and other clinical signs recorded in fish exposed to higher concentration of the onion bulb slurry in both bath and diet treatments shows that only lower dose could be used as feed additive. Although, few controlled and clinical studies have been conducted on the safety of fresh onion bulb in aquaculture, the findings of the present study suggest that safety could be dose-dependent, thus, at higher concentration; the onion bulb may not be safe. Strong positive relationship observed from the regression analysis in both BSLA and LD_{50} between concentration and percentage mortality further confirms the dose-effect potential and toxicity likely with increase concentration of the onion bulb.

Histopathology

The kidneys and livers of fish treated with *A. cepa* revealed varying degrees of vacuolar degenerations. The liver had vacuolar degeneration and necrosis of hepatocytes, congestion of the central veins and haemochromatosis in hepatocytes. The kidneys had vacuolar degeneration of tubular and glomerular epithelial cells (Table 7 and Figures 1 and 2). However, degenerations were more pronounced in bath treatments than feed.

Table 6
Lethal dose and lethal concentration of Allium cepa *slurry on experimental fish*

Treatments	Dose mg/ml	Log dose	Mortality	Mortality %	Probit	LC_{50}	R value
Dietary inclusion	200	2.3010	11	23.3	4.48	441.7 g/kg	0.8124
	150	2.1761	5	10	3.72		
	100	2	0	0	0		
	50	1.6989	0	0	0		
	25	1.3979	0	0	0		
Bath	5	0.6989	9	20	4.29	12.2g/L	0.9149
	3	0.4771	5	10	3.72		
	1.5	0.1761	5	10	3.72		
	0.7	-0.1549	0	0	0		
	0.4	-0.3979	0	0	0		

The effect of the onion bulb administered orally and through bath on the liver and kidney was determined in the study. The mild to moderate changes observed in these organs are reversible (Ozkurt et al., 2014). This suggests that the onion bulb has minimal detrimental effect when administered at low concentration. The degenerative changes observed in these organs might have resulted due to the presence of the phytochemical anthraquinone in the bulb (Chan & Lin, 2009).

Mild degenerations observed in *C. gariepinus* liver exposed to various concentrations of the fresh onion bulb via dietary inclusion and bath relative to the control liver confirmed the tendency for toxicity of the onion bulb (Borelli et al., 2009; Parton, 2000). Degenerative changes in the hepatocytes of the treatment group could be due to the presence of phytocompounds in the bulb of the onion which have been reported to have pathological responses in man (Abalaka et al., 2015). It could

Table 7
Histopathological changes observed in the organs of Clarias gariepinus sub-adult exposed to varying concentrations of whole Allium cepa *through feed and water*

	Histological signs	Route of administration										
		Feed (inclusion levels of onion in g/kg)					Bath (onion inclusion levels in g/L)					Control
		200	150	100	50	25	5	3	1.5	0.7	0.4	0 g/L
Kidney	Degeneration and necrosis of tubular epithelial cells	-	+	+	+	+	++	++	++	++	+	-
	Degeneration and necrosis of glomerular epithelial cells	-	+	+	+	+	+	+	+	+	+	-
	pigmentation of tubular epithelial cells	-	-	-		-	-	+	+	+	+	-
	haemosiderosis in the kidney	-	-	-		-	+	+	+	+	+	-
Liver	Vacuolar degeneration of the hepatocytes	+	++	++	++	++	++	+	++	++	+	-
	Congestion of the blood vessels	-	+	-	-	-	-	+	-	-	+	-
	Congestion of the central vein	-	+	-	+	-	+	+	+	+	+	-
	Necrosis of hepatocytes	-	-	-	-	-	-	-	-	-		-
	Hepatocytes infiltration by monoclear cells	-	-	-	-	-	-	-	-	-	-	-
	Haemosiderosis	-	-	-	-	-	-	-	+	+	-	-
	Encapsulation by fibrous connective tissue	-	-	-	-	-	-	-	-	-	-	-

Key: ++: = Present and distinct with morphological changes of histological signs on the organ and tissues. +=Present but less marked (mild) than usual. - = No lesion and morphological changes in organ and tissues

also be due to inability of the liver cells to metabolize fat during digestion resulting to small vacuoles of fat accumulating in the cytoplasm. However, vacuolar degeneration is reversible and mild degenerations may have no effect on cell function (Marcon et al., 2015).

The findings of this study corroborated the works of Al-Salahy and Mahmoud (2003) who reported hepatic vacuolar degeneration and necrosis in *Chrysichthys auratus* exposed to various concentrations of *Allium sativum* orally. However, Amrevuawho et al. (2016), reported on the ability of the onion bulb extracts to restore damaged liver cells of *C. gariepinus* exposed to *Pseudomonas aeruginosa*. Reason for these differences could be attributed to the state of health of the experimental fish.

The kidneys of treated experimental fish in this study showed various degrees of degeneration and necrosis of the tubular and glomerular epithelial cell in both routes of administration. The presence of haemosiderin laden macrophages was more in the fish exposed through bath. This might be due to direct accessibility of the extract to the circulatory system of the fish (blood)

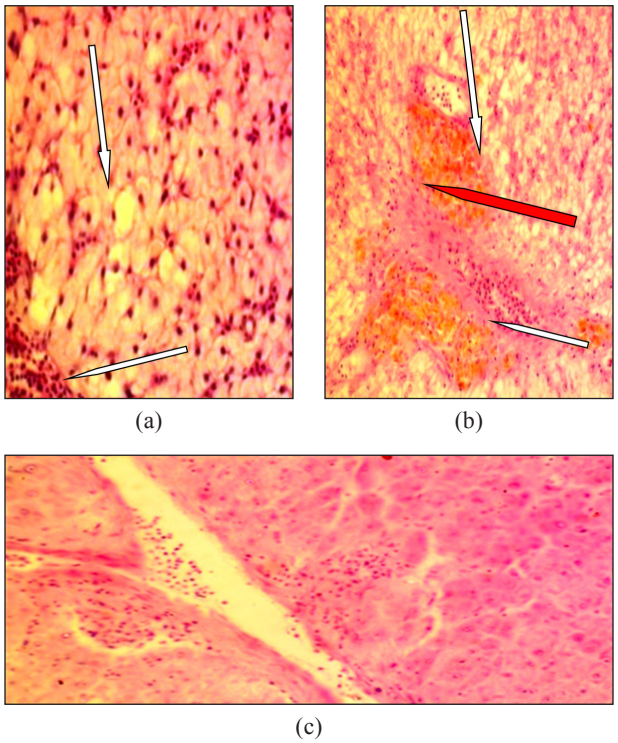

Figure 1. Liver sections in both routes of administration and control
Note. a. Section of the liver of fish treated orally with *A. cepa* showing severe vacuolar degeneration of hepatocytes (arrow) and congestion of sinusoid and central vein (arrow head) b. T8 Section of the liver of fish treated through bath with *A. cepa* showing vacuolar degeneration (arrow) and pigmentation of the hepatocytes (red arrow head) and congestion of the central vein (arrow head) c Section of the liver of fish not treated with *A. cepa* appearing apparently normal (x400; H 7 E)

via the gills during prolong bathing and subsequently enhance direct access to the kidney. Unlike group A in which dietary method was used, it was possible that most of the chemical compounds in the extract might have undergone biodegradation by the intestinal enzymes before entering the liver and the kidney resulting to mild effect in this group. Ola-Mudathir and Maduagwu (2014) and Wani et al. (2018) reported a contrary finding to that in this present study. Reasons could be due to differences in laboratory animal used and also could be due to the state of the animals.

Damage to tubular epithelial cells and haemosiderosis observed in the kidney can rarely result in kidney dysfunction and could be reversed (Ozkurt et al., 2014; Relia & Kaushik, 2010; Yatmark et al., 2016). Haemosiderosis could also be inherited as reported by Ozkurt et al. (2014) thus it could not be conclusive from this study that these degenerations were caused by the intake of the onion bulb.

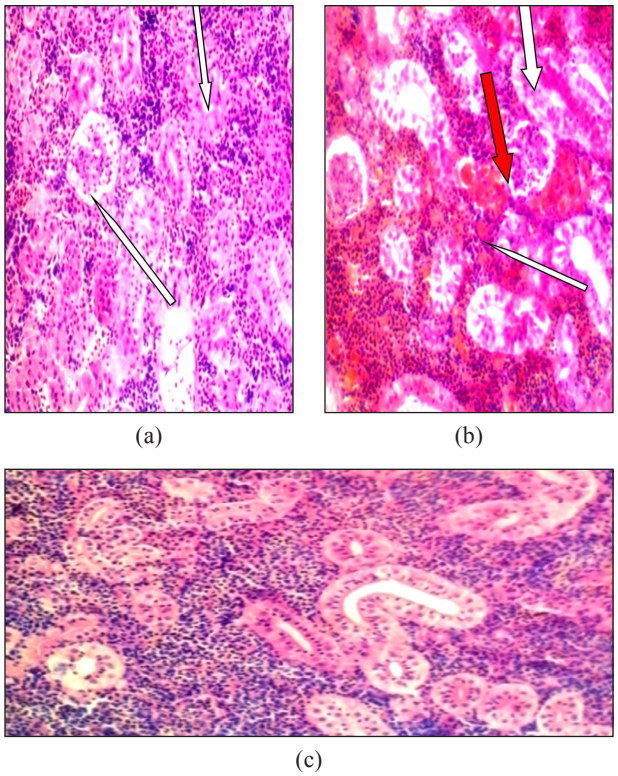

Figure 2. Comparative kidney sections in both routes of administration and control
Note. a. Treatment 3(100g/kg) Section of the kidney of fish treated with *A. cepa* showing degenerations and necrosis of tubular (arrow) and glomerular epithelial cells (arrow head) b. Treatment 6 (5g/L) Section of the kidney of fish treated with *A. cepa* show degeneration and necrosis of tubular (white arrow) and glomerular (red arrow) epithelial cells (arrow) with haemochromatosis (white arrow head) c. Section of the kidney of fish not treated with *A. cepa* appearing apparently normal (x400; H & E)

CONCLUSION

Allium cepa is safe in fish when administered in lower dose either through diet or bath exposure, since degenerative changes are reversible; however, the onion bulb extract can be harmful at high dose due to necrosis of hepatocytes and tubular epithelial cells.

ACKNOWLEDGMENTS

We thank I. O. Lawal and all staffs of the Biomedicine Department of the Forestry Research Institute of Nigeria for their help during the laboratory work. We also appreciate the effort of the laboratory staff of the Department of Veterinary Pathology of the Federal University of Agriculture, Abeokuta, Nigeria.

REFERENCES

Abalaka, S. E., Fatihu, M. Y., Ibrahim, N. D. G., & Ambali, S. F. (2015). Liver histopathological changes in *Clarias gariepinus* exposed to ethanol extract of *Adenium obesum* stem bark. *Journal of Morphological Sciences*, *32*(1), 22-28. doi: 10.4322/jms.069314

Abayomi, L. A., & Terry, L. A. (2009). Implications of spatial and temporal changes in concentration of pyruvate and glucose in onion (*Allium cepa* L.) bulbs during controlled atmosphere storage. *Journal of Science Food and Agriculture*, *89*(4), 683-687. doi: 10.1002/jsfa.3502

Adegbesan, S. I., Obasa, S. O., & Abdulraheem, I. (2017). Metabolic activities and health indices of African catfish (*Clarias gariepinus*) fed varying levels of *Zingiber officinale* root. *Journal of Applied Biology and Biotechnology*, *5*(4), 21-28.

Al-Salahy, M. B., & Mahmoud A. B. (2003). Metabolic and histological studies on the effect of garlic administration on the carnivorous fish *Chrysichthys auratus*. *Egyptian Journal of Biology*, *5*(1), 94-107.

Amrevuawho, M. O., Akinyemi, A. A., Oyewusi, A. J., Bankole, O. M., & Ezeri, G. N. O. (2016). Effects of onion (*Allium cepa*) and chloramphenicol on haematological parameters, histopathology and survival of catfish C*larias gariepinus* (Burchell, 1822) sub-adult infected with *Pseudomonas aeruginosa*. *Journal of Veterinary Science and Technology*, *11*(1), 1-12.

Association of Official Analytical Chemists. (2005). *Official methods of analysis of the Association of Analytical Chemists International* (18th ed.). Washington, USA: MD Publishing.

Augusti, K. T. (1996). Therapeutic values of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.). *Indian Journal of Experimental Biology*, *34*(7), 634-40. doi: 10.19046/abp.v03i04.01

Ashwini, M., Balaganesh, J., Balamurugan, S., Murugan, S. B., & Sathishkumar, R. (2013). Antioxidant activity in *in vitro* and *in vivo* cultures of onion varieties (Bellary and CO 3). *Food and Nutrition Sciences*, *4*, 918-923. doi: 10.4236/fns.2013.49119

Bastos, M. L. A., Lima, M. R. F., Conserva, L. M., Andrade, V. S., Rocha, E. M. M., & Lemos, R. P. L. (2009). Studies on the antimicrobial activity and brine shrimp toxicity of *Zeyheria tuberculosa* (Vell.) Bur. (Bignoniaceae), extracts and their main constituents. *Annals of Clinical Microbiology and Antimicrobials*, *8*(1), 16. doi: 10.1186/1476-0711-8-16

Batta, H. (2012). Press coverage of traditional medical practice in Nigeria. *Journal of Communications*, *3*(2), 75-89.

Bello, O. S., Olaifa, F. E., Emikpe, B. O., & Ogunbanwo, S. T. (2012). The effect of walnut (*Tetracarpidium conophorum*) leaf and onion (*Allium cepa*) bulb residues on the tissue bacteriological changes of *Clarias gariepinus*

juvenile. *Bulletin des Santé et Production Animales en Afrique*, *60*(2), 205-212.

Bhattacharjee, S., Sultana, A., Sazzad, M. H., Islam, M. A., Ahtashom, M., & Asaduzzaman, M. (2013). Analysis of the proximate composition and energy values of two varieties of onion (*Allium cepa* L.) bulbs of different origin: A comparative study. *International Journal of Nutrition and Food Sciences*, *2*(5), 246-253. doi:10.11648/j.ijnfs.20130205.16

Borelli, V., Lucioli, J., Fernando, H. F., Giovana, H. P., Fleck, R. J., Davi, T. S., & Aldo, G. (2009). Fatal onion (*Allium cepa*) toxicosis in water buffalo (*Bubalus bubalis*). *Journal of Veterinary Diagnostic Investigation*, *21*(3), 402-405. doi: 10.1177/104063870902100321

Carballo, J. L., Hernández-Inda, Z. L., Pérez, P., & García-Grávalos, M. D. (2003). A comparison between two brine shrimp assays to detect *in vitro* cytotoxicity in marine natural products. *BMC Biotechnology*, *2*(1), 17-21. doi: 10.1186/1472-6750-2-17

Chan, K., & Lin, T. X. (2009). Chapter 48 - Treatments used in complementary and alternative medicine. *Side Effects of Drugs Annual*, *31*, 745-756. doi: 10.1016/S0378-6080(09)03148-1

Duncan, D. B. (1955). Multiple range and multiple F tests. *Biometrics*, *11*(1), 1-42.

El-Sayed, Y. S., El-Okle, O. S. M., Hassan, S. M. H., & Bakir, N. M. A. (2015). Poisoning of cattle feeding on Allium ampeloprasum (Egyptian kurrat). *Journal of Veterinary Science and Medical Diagnosis, 4*(4), 1000170-1000173. doi:10.4172/2325-9590.1000170

Food and Agricultural Organization. (2019). Nigeria at a glance. Retrieved April 03, 2019, from http://www.fao.org/nigeria/fao-in-nigeria/nigeria-at-a-glance/en/

Finney, D. J. (1952). *Probit analysis: A statistical treatment of the sigmoid response curve*. Cambridge, United Kingdom: Cambridge University Press.

Gazuwa, S. Y., Makanjuola, E. R., Jaryum, K. H., Kutshik, J. R., & Mafulul, S. G. (2013). The phytochemical composition of *Allium cepa/ Allium sativum* and the effects of their aqueous extracts (cooked and raw forms) on the lipid profile and other hepatic biochemical parameters in female albino Wistar rats. *Asian Journal of Experimental Biological Sciences*, *4*(3), 406-410.

Griffiths, G., Trueman, L., Crowther, T., Thomas, B., & Smith, B. (2002). Onions - A global benefit to health. *Phytotherapy Research, 16*(7), 603-615.

Iloki-Assanga, S. B., Lewis-Luján, L. M., Lara-Espinoza, C. L., Gil-Salido, A. A., Fernandez-Angulo, D., Rubio-Pino, J. L., & Haines, D. D. (2015). Solvent effects on phytochemical constituent profiles and antioxidant activities, using four different extraction formulations for analysis of *Bucida buceras* L. and *Phoradendron californicum. BMC Research Notes*, *8*(1), 396-410. doi: 10.1186/s13104-015-1388-1

Johansen, R., Needham, J. R., Colquhoun, D. J., Poppe, T. T., & Smith, A. J. (2006). Guidelines for health and welfare monitoring of fish used in research. *Laboratory Animals*, *40*(4), 323–340.

Kirk, S. R., Sawyer, R., & Egan, H. (1991). Pearson's composition and analysis of foods (9th ed.). Singapore: Longman Publishers.

Kuete, V. (2017). *Medicinal spices and vegetables from Africa: Therapeutic potential against metabolic, inflammatory, infectious and systemic diseases*. Cambridge, USA: Academic Press.

Manilal, A., Sujith, S., Kiran, G. S., Selvin, J., Shakir, C., Gandhimathi, R., & Lipton, A. P. (2009). Antimicrobial potential and seasonality of red algae collected from the Southwest coast of India tested against shrimp, human and phytopathogens. *Annals of Microbiology*, *59*(2), 207-219.

Marcon, L., Bazzoli, N., Mounteer, A. H., & dos Anjos Benjamin, L. (2015). Histological and histometric evaluation of the liver in *Astyanax Bimaculatus* (Teleostei: Characidae), exposed to different concentrations of an organochlorine insecticide. *Anatomical Record*, *298*(10), 1754–1764. doi: 10.1002/ar.23196

Martini, R., & Lindberg, C. (2013). *Fishing for Tomorrow: Managing fisheries for sustainable development*. Retrieved April 03, 2019, from http://www.oecd.org/gov/pcsd/002013401_Coherence%20for%20Development_Dec_PDFX_WEB.pdf

National Bureau of Statistics. (2019). *Nigerian gross domestic product report: Q4 and full year 2018*. Abuja, Nigeria: NBS.

Nebedum, J., Ajeigbe, K., Nwobodo, E., Uba, C., Adesanya, O., Fadare, O., & Ofusori, D. (2009). Comparative study of the ethanolic extracts of four Nigerian plants against some pathogenic microorganisms. *Research Journal of Medicinal Plants*, *3*(1), 23-28. doi: 10.3923/rjmp.2009.23.28

Nicastro, H. L., Ross, S. A., & Milner, J. A. (2015). Garlic and onions: Their cancer prevention properties. *Cancer Prevention Research*, *8*(3), 181-189. doi: 10.1158/1940-6207.CAPR-14-0172

Obaroh, I. O., Keta, J. N., & Nwaogu, J. (2018). Growth performance of *Clarias gariepinus* fed crude extracts of *Allium cepa*, *Moringa oliefera* and *Vernonia amygdalina*. *Asian Journal of Animal Science*, *12*(1), 36-41. **doi:** 10.3923/ajas.2018.36.41

Obioha, U. E., Suru, S. M., Ola-Mudathir, K. F., & Faremi, T. Y. (2009). Hepatoprotective potentials of onion and garlic extracts on cadmium-induced oxidative damage in rats. *Biological Trace Element Research*, *129*(1-3), 143-156. doi: 10.1007/s12011-008-8276-7

Ogbonna, O. J., Udia, P. M., Abe, P. N., Omoregha, C. U., & Anele, E. I. (2016). Phytochemical and proximate analysis, mineral and vitamin compositions of *Allium cepa* bulb extract. *Advances in Biomedicine and Pharmacy*, *3*(4), 181-186. doi: 10.2016/adbp

Ohikhena, F. U., Wintola, O. A., & Afolayan, A. J. (2016). Toxicity assessment of different solvent extracts of the medicinal plant, *Phragmanthera capitata* (Sprengel) balle on brine shrimp (*Artemia salina*). *International Journal of Pharmacology*, *12*(7), 701-710. **doi:** 10.3923/ijp.2016.701.710.

Ola-Mudathir, K. F., & Maduagwu, E. N. (2014). Antioxidant effects of methanol extract of *Allium cepa* Linn. on cyanide-induced renal toxicity in male Wistar rats. *Nigerian Journal of Physiological Sciences*, *29*(2), 147-151.

Opiyo, M. A., Marijani, E., Muendo, P., Odede, R., Leschen, W., & Charo-Karisa, H. (2018). A review of aquaculture production and health management practices of farmed fish in Kenya. *International Journal of Veterinary Science and Medicine*, *6*(2), 141–148. doi:10.1016/j.ijvsm.2018.07.001

Ozkurt, S., Acikalin, M. F., Temiz, G., Akay, O. M., & Soydan, M. (2014). Renal hemosiderosis and rapidly progressive glomerulonephritis associated with primary hemochromatosis. *Renal Failure*, *36*(5), 814-6. doi: 10.3109/0886022X.2014.892391

Parton, K. (2000). Onion toxicity in farmed animals. *New Zealand Veterinary Journal*, *48*(3), 89-89. doi: 10.1080/00480169.2000.36168

Patil, M. V., & Patil, D. A. (2007). Some herbal remedies used by the tribals of Nasik district, Maharashtra. *Natural Product Radiance*, *6*(2), 152-157.

Pulvertaft, R. J. V. (1950). Museum techniques: A review. *Journal of Clinical Pathology*, *3*(1), 23.

Relia, N., & Kaushik, C (2010). Renal hemosiderosis: A case of black kidneys causing renal failure. *Journal of Postgraduate Medicine*, *56*(3), 216-217. doi: 10.4103/0022-3859.68635.

Salami, H. A, John, A. I., & Ekanem, A. U. (2012). The effect of aqueous preparation of *Allium cepa* (onion) and *Allium sativa* (garlic) on erythrocyte osmotic fragility in wistar rats: *In vivo* and *in vitro* studies. *Nigerian Journal of Physiological Sciences*, *27*(1), 29-34.

Saleh, N. E., Michael, F. R., & Toutou, M. M. (2015). Evaluation of garlic and onion powder as phyto-additives in the diet of sea bass (*Dicentrarcus labrax*). *Egyptian Journal of Aquatic Research*, *41*(2), 211-217.

Santosh, C. H., Attitalla, I. H., & Mohan, M. M. (2013). Phytochemical analysis, antimicrobial and antioxidant activity of ethanolic extract of *Vernonia anthelmintica*. *International Journal of Pharmacy and Biological Sciences*, *4*(1), 960-966.

Syahmi, A. R. M., Vijayarathna, S., Sasidharan, S., Latha, L. Y., Kwan, Y. P., Lau, Y. L., … Chen, Y. (2010). Acute oral toxicity and brine shrimp lethality of *Elaeis guineensis* Jacq. (oil palm leaf) methanol extract. *Molecules*, *15*(11), 8111–8121. doi: 10.3390/molecules15118111

Ugwu, E. C., & Omale, J. (2011). Comparative effects of aqueous garlic (*Allium sativum*) andonion (*Allium cepa*) extracts on some haematological and lipid indices of rats. *Annual Review and Research in Biology*, *1*(3), 37-44.

Wani, A. A., Sikdar, M., & Zube, S. M. (2018). Nephroprotective action of taurine and garlic extract against copper sulphate induced renal toxicity in *Clarias gariepinus*. *International Journal of Pharmacy and Biological Sciences*, *8*(1), 2321-3272.

Widyawati, P. S., Budianta, T. D. W., Kusuma, F. A., & Wijaya, E. L. (2014). Difference of solvent polarity to phytochemical content and antioxidant activity of *Pluchea indicia* less leaves extracts. *International Journal of Pharmacognosy and Phytochemical Research*, *6*(4), 850-855

Yarahmadi, P., Miandare, H. K., & Hoseinifar, S. H. (2016). Hemato-immunological and serum biochemical parameters, intestinal histomorphology and growth performance of rainbow trout (*Oncorhynchus mykiss*) fed dietary fermentable fiber (Vitacel®). *Aquaculture Nutrition*, *22*(5), 1134-1142. doi: 10.1111/anu.12388

Yatmark, P., Morales, N. P., Chaisri, U., Wichaiyo, S., Hemstapat, W., Srichairatanakool, S., & Fucharoen, S. (2016). Iron distribution and histopathological study of the effects of deferoxamine and deferiprone in the kidneys of iron overloaded β-thalassemic mice. *Experimental and Toxicologic Pathology*, *68*(8), 427-34. doi: 10.1016/j.etp.2016.06.006