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African Journal of Biochemistry Research

January-March 2020
ISSN 1996-0778
DOI: 10.5897/AJBR
www.academicjournals.org

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Vol. 14(1), pp. 1-4, January-March 2020
DOI: 10.5897/AJBR2019.1063
Article Number: CAC4DA362702
ISSN 1996-0778
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African Journal of Biochemistry Research

Full Length Research Paper

Prevalence of dyslipidemia in a Burkinabe military population

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Received 4 November, 2019; Accepted 8 January, 2020

The objective of this study was to determine the prevalence of dyslipidemia in soldiers received at the laboratory of the Medical Center Camp General Aboubacar Sangoulé Lamizana (CMCGASL) for a medical visit. This is a prospective study of 224 military personnel assigned to peacekeeping missions outside Burkina Faso. The study was conducted over the period of September 2017 to November 2017. Included in the study were the military with at least one lipid status parameter. Total cholesterol, HDL cholesterol, LDL cholesterol as well as triglycerides were measured using enzymatic methods on a KONELAB20® Biochemistry machine. The prevalence of dyslipidemia in the study population was 41.96%. The average age of our patients was 39.49 years and the most represented age group was 30 to 57 years old. The prevalences of hypercholesterolemia, hyperlipidemia, hypertriglyceridemia and hypolipidemia were respectively: 3.12, 33, 93, 4.91 and 0%. The dyslipidemias were strongly correlated with hypertension. The prevalence of dyslipidemia in the military was high. These results demonstrate the value of conducting an epidemiological survey on cardiovascular risk factors in the Burkinabe armed forces.

Key words: Dyslipidemia, total cholesterol, HDL cholesterol, LDL cholesterol, atherogenicity index.

INTRODUCTION

Dyslipidemias represent a public health problem with a prevalence that exceeds 30% in Western countries. In sub-Saharan Africa, prevalence varies by region and rates of more than 50% have been found in Ghana, Nigeria and 39.30% in Senegal (Fatou et al., 2016). In Burkina, despite the frequency of cardiovascular diseases, data on the prevalence of risk factors are scarce. Studies in Burkina Faso and other sub-Saharan African countries show dyslipidemia in 20-90% of diabetic patients (Guira et al., 2018). The search for these risk factors and their adequate management could help prevent cardiovascular diseases. The progression of cardiovascular diseases is linked to several factors, among which the development of certain factors, known as "Cardiovascular risk factors". These factors are smoking, diabetes, high blood pressure (hypertension),

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Table 1. Socio-demographic characteristics of the study population.

Variable	Total n (%)	Dyslipidemia	
		Yes, n (%)	No, n (%)
Sex			
Male	215(96)	93(43.25)	122(56.74)
Female	9(4)	1(11.11)	8(88.89)
Age (years)			
<40	121 (54.01)	18 (14.88)	103 (85.12)
40-50	59 (26.34)	39 (66.10)	20 (33.90)
>50	35 (15.62)	29 (82.86)	6 (17.14)
unspecified	9 (4.01)	8 (88.88)	1
Clinical diagnoses			
high blood pressure	45/198 (22.72)	33 (73.33)	12 (26.66)
Overweight- obesity	81/169 (47.93)	30 (37.03)	51 (62.96)
Hyperglycemia	49/191 (25.65)	25 (51.02)	24 (48.98)

Table 2. Prevalence of dyslipidemia.

Prevalence	Effective	Percentage (%)
Dyslipidemia	94	41.96
Hypercholesterolemia	07	3.12
Hyperlipidemia	76	33.93
Hypertriglyceridemia	11	4.91
Hypolipidemia	0	0
Atherogenicity index	191	85.27

dyslipidemia, obesity, sex, family history of cardiovascular disease, nutritional factors, and sedentary lifestyle (Hajar, 2017). The lipid balance, a simple examination accessible to all laboratories is a step in this prevention strategy. Thus, in this work, we set ourselves the objective of assessing the prevalence of dyslipidemia in soldiers placed in an external mission position received at the laboratory of the Medical Center of the Camp of General Aboubacar Sangoulé Lamizana (CMCGASL).

MATERIALS AND METHODS

This is a prospective study of 224 military personnel aged 25 to 57 years designated for peacekeeping missions outside Burkina Faso. The study was carried out over the period from September 2017 to November 2017. Lipid biomarkers were evaluated. Total cholesterol, HDL cholesterol, LDL cholesterol, and triacylglycerol were measured using enzymatic methods on Biochemistry KONELAB20®. The dyslipidemia was defined according to the criteria of The National Cholesterol Education Program (NCEP) (Expert Panel on Detection, 2001): Cholesterolemia (total cholesterol> 2 g/L (11.11 mmol / L), hypolipidemia (HDL cholesterol) <0.4 g/L (2.22 mmol/L), hypertriglyceridemia (triacylglycerol> 1.5 g/L (8.3 mmol / L) and - hyperlipidemia (LDL cholesterol > 1.3 g/L (7, 22 mmol/L). The atherogenicity index (IA) is the ratio of total cholesterol to HDL cholesterol: Male: <5 Female: <4.5. The classification according to the Body Mass Index (BMI) was made according to WHO recommendations in the following intervals: Skinny for a BMI <18.5 kg/m²; Normal BMI (for 18.5 to <BMI ≤ 24.9 kg/m²); Overweight (for BMI of 25 to <BMI ≤ 29.9 kg/m²) and Obese (for BMI ≥ 30 kg/m²). The formula for BMI is weight in kilograms divided by height in meters squared. If height has been measured in centimeters, divide by 100 to convert this to meters. Hyperglycemia was defined as Fasting plasma glucose ≥ 7.0 mmol/L (126 mg/dl). According to WHO recommendations, normal systolic blood pressure is less than 140 mmHg. The mean value of the diastolic blood pressure is less than 90 mmHg. The data was collected on Excel 2016 and analyzed by IBM SPPS 22.

RESULTS AND DISCUSSION

The current study covered 224 subjects. The average age of the patients under this study was 39.49 with extremes of 25 to 57 years. Our sample consisted of nine female patients and 215 male patients (Table 1). It was observed that 14 (6.25%) were obese patients and 67 (29.9%) were overweight patients (Table 2). The mean value of the systolic blood pressure of the patients was 127.6 mmHg. The mean value of patients' diastolic blood

Table 3. Correlation between dyslipidemia and other cardiovascular risk factors (hypertension, diabetes, obesity).

Dyslipidemia	Yes	No	RR
High blood pressure			
Yes (n=45)	33	12	2.55
No (n=153)	44	109	
Hyperglycemia			
Yes (n=49)	25	24	0.90
No (n=142)	65	77	
Overweight, Obesity			
Yes (n=81)	30	51	0.49
No (n=88)	66	22	

pressure was 81.7 mmHg. High blood pressure was found in 45 (22.72%) patients (Table 1). The average blood glucose was 5.38 mmol /L. Hyperglycemia was found in 49 patients. The prevalence of dyslipidemia in the study population is 41.96%. The prevalences of hypercholesterolemia,hyperlipidemia,hypertriglyceridemia and hypolipidemia were respectively: 3.12, 33.93, 4.91 and 0% (Table 2). The distribution of age-related dyslipidemias shows that subjects over 50 are more exposed. Multivariate analysis between the dyslipidemia variable and the other cardiovascular risk factors (Table 3) shows an association between dyslipidemias and high blood pressure (RR = 2.55).

The study of 224 soldiers showed that people over 50 were the most affected (Table 1). These data are confirmed by a study carried out in France in 2006 which found a prevalence of dyslipidemia of about 67% in the age group of subjects aged 55 to 74 and reported by Fatou et al. (2016). Oghagbon and Okesina (2006) and Gao et al. (2012) found an increase in the incidence of dyslipidemia with age in Nigeria and China, respectively. In this study, it was 96% of men in our sample. Balaka et al. (2017) found 67.8% of male patients. Dominique et al. (2014), Pessinaba et al. (2013), Scheidt-Nave et al. (2013), Adébayo et al. (2016) respectively found 62.5, 69, 60.5 and 74.6% of female patients. This predominance of men in our study could be explained because it is a military population. Indeed, although there are women in the Burkinabe army, their number is small. The prevalence of dyslipidemia in this study is high (41.96%). The major role of dyslipidemia in the genesis of cardiovascular disease has been established by large studies in population cohorts, particularly in the United States (Robert and Nelson, 2013) and in Europe (Julian et al., 2017). The results of this are comparable to those found by Tiahou et al. (2010) and also corroborate the prevalence found in Senegalese studies (Pessinaba et al., 2013; Thiombiano et al., 2016; Fatou et al., 2016) and are similar to prevalences observed in industrialized countries that exceed 30% (Ferrieres et al., 2005; Scheidt-Nave et al., 2013; Tóth et al., 2012). In this study, hyperlipidemia is the most common dyslipidemia (33.93%) followed closely by hypertriglyceridemia (4.91%).This predominance of hyperlipidemia has also been reported by the work of Fatou Cissé et al. (2016) as well as Erem et al. (2008) in Turkey. A Togolese study found total hypercholesterolemia (25.91%) followed by hyperlipidemia (24.3%) as part of the annual health check of the staff of the Post Office of Togo (Balaka et al., 2017). Hyperlipidemia was present in 29% of patients in the Guira et al. (2018) in the newly diagnosed type 2 diabetic at the Yalgado Ouedraogo University Hospital Center in Ouagadougou. Agboola-Abu et al. (2000) in Nigeria found a lower frequency of hyperlipidemia (21.4%). Tian et al. (2015) in his study in China reported more than double our prevalence (66%). The results of this study differ from those found in Algeria (14.3%) (Yahia-Berrouiguet et al., 2009). However, most authors have found a predominance of hypercholesterolemia (Tiahou et al., 2010; Khader et al., 2010; Baragou et al., 2012; Scheidt-Nave et al., 2013; Micah and Nkum, 2012). Hyperlipidemia is almost always associated with hypercholesterolemia. This study is distinguished by the fact that hyperlipidemia is not correlated with hypercholesterolemia because LDL values were slightly above normal values and HDLs close to low values, therefore, total cholesterol values are close to normal; limit values without exceeding them. The mean value of the atherogenicity index (TC / HDL-c) in this study population was 9.16, which is higher than normal. The atherogenicity index is high in 85.27% of the study population. In the study of Adébayo et al. (2016), the atherogenicity index was 18.44% higher in people living with HIV with an Atherogenicity Index average of 6.61, which is also higher than normal. The prevalences of hypertriglyceridemia and hypoHDLemia were 4.91 and 0%, respectively. This order of frequency is different from the study of Ferrieres et al. (2005) and Fatou et al. (2016); hypolipidemia was the second most common lipid abnormality. The prevalence of hypertriglyceridemia in

this study differs from that of Guira et al. (2018) with a prevalence of 30.0% in type 2 diabetic population. According to some studies, the influence of genetic, ethnic, and environmental factors may be responsible for a lower frequency of hypertriglyceridemia in black subjects. In this study, no case of hypolipidemia was recorded. This prevalence differs from that of Guira et al. (2018) who observed hypolipidemia in 61.2% of patients which is consistent with the profile of HDL-c described in the classic lipid profile of type 2 diabetic patients. A frequency of 69 .6% was reported by Tian et al. (2015) in China. The exploitation of these data showed an association between dyslipidemia and other risk factors such as hypertension (RR = 2.55) (Table 3). This association was also found in the study by Pessinaba et al. (2013) and Fatou et al. (2016). In the case of obesity, an association with dyslipidemia was not found. This could be explained by the fact that it is a military population that regularly plays sports.

Conclusion

This study highlights a high prevalence of dyslipidemia in the military. Findings may be underestimated as most of these patients were already in contact with the health system and maybe on cholesterol-lowering therapy. This demonstrates the value of conducting an epidemiological survey of cardiovascular risk factors at the national level.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Adébayo A, Albert CD, Angelo CA, Jules G, Moutawakilou G, Armand W, Léopold C, Colette A, Djimon MZ, François D (2016). Profil lipidique des personnes vivant avec le VIH sous antirétroviral suivis au Centre Hospitalier Universitaire Ouémé-Plateaux (CHU-O/P) de Porto-Novo, Bénin. International Journal of Biological and Chemical Sciences 10(5):2175-2182.

Agboola-Abu CF, Ohwovoriole AE, Akinlade KS (2000). The effect of glycaemic control on the prevalence and pattern of dyslipidemia in Nigerian patients with newly diagnosed non-insulin dependent diabetes mellitus. West African Journal of Medicine 19(1):1-5.

Balaka A, Djibril MA, Tchamdja T, Djagadou KA, Mossi E, Nemi KD (2017). Cardiopathies ischémiques et dyslipidémies en milieu professionnel postal au Togo. Revue Africaine de Médecine Interne 4(1):7-9.

Baragou S, Djibril M, Atta B, Damorou F, Pio M, Balogou A (2012). Prevalence of cardiovascular risk factors in an urban area of Togo: a WHO STEPS-wise approach in Lome, Togo. Africa Cardiovascular Journal of Africa 23(6):309-312.

Dominique D, Abdou SM, Fatou AD, Modou J, Arame N, Adama K, Alassane D, Meissa T (2014). Lipid profile frequency and the prevalence of dyslipidemia from biochemical tests at Saint Louis University Hospital in Senegal. Pan African Medical Journal 17:75.

Erem C, Hacihasanoglu A, Deger O, Kocak M, Topbas M (2008). Prevalence of dyslipidemias and associated risk factors among Turkish adults: Trabzon Lipid Study. Endocrine 34(13):36- 51.

Expert Panel on Detection (2001). Evaluation, and Treatment of High Blood Cholesterol in Adults.Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). The Journal of the American Medical Association 285(19):2486-97.

Fatou C, Fatou DA, Alassane D, Abdou SM, Arame N, Abdourahmane S, Souleymane T, Dominique D, Gaston NS, Niama DS, Méissa T (2016). Prévalence des dyslipidémies au laboratoire de biochimie du CHU Aristide le Dantec de Dakar, Sénégal. The Pan African Medical Journal 25:67.

Ferrieres J, Ruidavets JB, Perret B, Dallongeville J, Arveiler D, Bingham A, Amouyel P, Haas B, Ducimetiere P (2005). Prévalence des dyslipidémies dans un échantillon représentatif de la population française. Archives des Maladies du Coeur et des Vaisseaux 98(2):127-32.

Gao Y, Zhong XN, Yang YH, Tian KC (2012). Plasma lipid level and incidence of dyslipidemia in workers of Chongqing enterprises and institutions. Zhonghua Xin Xue Guan Bing Za Zhi 40(5):432-435.

Guira O, Nagalo A, Tiéno H, Zoungrana L, Bognounou R , Tondé A , Traoré R, Sakandé J, Drabo J Y (2018). LDL cholestérol chez le diabétique de type 2 nouvellement diagnostiqué au Centre Hospitalier Universitaire Yalgado Ouédraogo, Ouagadougou (Burkina Faso). Revue Africaine de Médecine Interne 5(2):37-42.

Hajar R (2017). Risk factors for coronary artery disease: historical perspectives. Heart Views 18(3):109–114.

Julian PH, José RB, Carine R, Jean D, Guy DB, Eliseo G, Joep P, David H, Karin MH, Claudio B (2017). Prevalence and treatment of atherogenic dyslipidemia in the primary prevention of cardiovascular disease in Europe: EURIKA, a cross-sectional observational study. BioMed Central Cardiovascular Disorders 17:160.

Khader YS, Batieha A, El-Khateeb M, Al Omari M, Ajlouni K (2010). Prevalence of dyslipidemia and its associated factors among Jordanian adults. Journal of Clinical Lipidology 4(1):53-58.

Micah FB, Nkum BC (2012). Lipid disorders in hospital attendants in Kumasi, Ghana. Ghana Medical Journal 46(1):14-21.

Oghagbon EK, Okesina AB (2006). The pattern of some risk factors for cardiovascular disease in untreated Nigerian hypertensive patients. West African Journal of Medicine 25(3):190-194.

Pessinaba S, Mbaye A, Yabéta GAD, Harouna H et al. Pessinaba S, Mbaye A , Yabéta GAD, Harouna H, Sib AE, Kane AD, Bodian M, Ndiaye MB, Mbaye-Ndour M, Niang K, Diagne-Sow D, Diack B, Kane M, Diao M, Mathieu JBS, Kane A (2013). Enquête de prévalence des facteurs de risque cardiovasculaire en population générale à Saint-Louis (Sénégal). Annales de Cardiologie Angeiologie 62(4):253-258.

Robert H, Nelson MD (2013). Hyperlipidemia as a Risk Factor for Cardiovascular Disease. Primary Care: Clinics in Office Practice 40(1):195-211.

Scheidt-Nave C, Du Y, Knopf H, Schienkiewitz A, Ziese T, Nowossadeck E, Gößwald A, Busch MA (2013). Prevalence of dyslipidemia among adults in Germany: results of the German Health Interview and Examination Survey for Adults (DEGS 1). Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz 56(5-6):661-667.

Tiahou G, Deret K, Monde A. Agniwo Camara-Cissé M, Djohan Y, Djessou P, Sess D (2010). Fréquence des bilans lipidiques et prévalence des dyslipidémies au laboratoire de biochimie du chu de Cocody. Journal Pharmaceutical and Biological Sciences 11(2):60-65.

Tian J, Chen H, Jia F, Yang G, Li S, Li K, Zhang L, Wu J, Liu D (2015). Trends in the levels of serum lipids and lipoproteins and the prevalence of dyslipidemia in adults with newly diagnosed type 2 diabetes in the Southwest Chinese Han Population during 2003–2012. International Journal of Endocrinology pp. 1-7.

Tóth PP, Potter D, Ming EE (2012). Prevalence of lipid abnormalities in the United States: the National Health and Nutrition Examination Survey. Journal of Clinical Lipidology 6(4):325-330.

Yahia-Berrouiguet A, Benyoucef M, Meguenni K, Faivre B, Brouri M (2009). Enquête sur la prévalence des facteurs de risque de maladies cardiovasculaires à Tlemcen (Algérie). Diabetes and Metabolism 35(1):42-43.

Vol. 14(1), pp. 5-17, January-March 2020
DOI: 10.5897/AJBR2019.1051
Article Number: 5C018F763079
ISSN 1996-0778
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Full Length Research Paper

The effect of aqueous extract of *zest of citrus sinensis* (AEZCs) on cadmium chloride induced liver toxicity in wistar rats

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Received 21 August, 2019; Accepted 10 February, 2020

The potency of the peels of *Citrus sinensis* against cadmium induced liver damage has not been explored in our environment. 48 wistar rats were used for this study. The animals were randomly divided into eight (8) groups of five (5) rats each. Group A was the positive control and received 5 mg/kg body weight (bw) of cadmium chloride ($CdCl_2$) intraperitoneally as a single dose. Groups B and C received the aqueous extract of zest of citrus sinensis (AEZCS) at a low doses of 10 and 40 mg/kg bw respectively. Groups D and E received cadmium chloride, followed by low and high doses of AEZCS respectively. Groups F and G received low and high doses of AEZCS followed by CdCl2 while group H served as the normal control. Liver enzymes (AST, ALT and ALP) and serum total proteins were analyzed. The results showed significant (P<0.05) differences in the mean values of LV/BW, ALT, AST, total proteins, serum dismutase (SOD) and malondialdehyde (MDA) when the positive control group was compared with the normal control group (P<0.05). Histological sections of the negative control groups were significantly different from the positive control group but not from the groups treated with AEZCS at the high doses. Thus, AEZCS had ameliorative and protective health benefits at the high dose of 40mg/kg body weight.

Key words: *Citrus sinensis*, oxidative stress, cadmium chloride, hepatotoxicity.

INTRODUCTION

Citrus is widely grown in Nigeria and many other tropical and subtropical regions (Piccinelli et al., 2008) In terms of

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ranks, citrus ranks after banana as the world second fruit crop with more than 108 million tons (FAO Statistics, 2007). It originated from Southern China where it has been cultivated for many years but it is today grown commercially in tropical, semi tropical and some warm temperate regions to become the most widely planted fruit tree in the world (Nicolosi et al., 2000). Various existing species of citrus are of useful benefits. They are *Citrus limon* (lemon), *medica* (citron), *aurantium (s*our orange), *C. paradisi* (grapefruit), *C. reticulata* (mandalain ,tangerine), *C. clementina an*d *C.sinensis* (sweet orange). Citrus sinensis is also referred to as sweet orange and belongs to the family *Rutacea* with a life span of over years (Geeta and Kalidhar 2010). *Citrus sinensis* is called sweet orange in English; the Yorubas, Igbos and Hausas call it *Osan, oroma and lemu* respectively (Etebu and Nwauzoma, 2014). The height of orange tree is generally 9-10 m, with large spines on the branches. Anatomically, the fruit consists of two distinct regions; the pericarp also called the peel, skin, zest or rind and the endocarp or the pulp and juice sacs (Geeta and Kalidhar 2010). The skin consists of an epidermis of epicuticular wax with numerous small aromatic oil glands that gives it its particular smell (Favela–Hernandez et al., 2016). The citrus zest or peels contain important phytochemical and nutritional elements such as calcium, coumarins, peptides, vitamin C (ascorbic acids), phenolic antioxidants: flavonoids, alkaloids, saponins, tannins, hesperidin and naringin, beta– cryptoxanthin (the pigment that gives oranges gloss paint) (Answar, 2014). While the flesh and nectar of these citrus sinensis fruits are usually consumed as food, herbalists have their peels as medicine for numerous maladies throughout history. The citrus peel extract is used for nurturing breast when added to soybean oil, honey, propolis and other substances. There are researches on the effects of citrus sinensis peel against toxicity induced by xenobiotics or mutagenic agents (Marc and George 1997).

Cadmium is a relatively rare element (0.2 mg/kg in the earth crust) that is not found in the pure state in nature. It occurs mainly in association with the sulphides ores of zinc, lead and copper. Cadmium compounds have varying degrees of solubility of the specific cadmium compound as well as its exposure concentration and route. Cadmium is more efficiently absorbed from the lungs than from the gastrointestinal tract (ATSDR, 1989). As a multi– target toxicant, it is transplanted in the blood and widely distributed in the body then accumulates primarily in the liver and kidneys after absorption. Its burden tends to increase in a linear fashion up to 50-60 years of age after which the burden remains somewhat constant (Goyer, 1991). Cadmium is a xenobiotics toxicant of environmental and occupational concern and it has been classified as ahuman carcinogen; inhalation of cadmium has been implicated in the development of emphysema, pulmonary fibrosis but the detailed mechanism by which cadmium induced adverse biological effect might be its central role in xenobiotics metabolism (Shin et al., 2003). Cadmium and many xenobiotics (drugs and environmental chemicals) are capable of causing some degree of liver toxicity. In US, xenobiotics–induced liver toxicity is implicated in 2-5% of the hospitalizations for jaundice; an estimated 15 -30% of the fulminant liver failure, and 40% of the acute cases in individuals older than 50 (Marc and George 1997). Fortunately, most drug-induced liver injuries resolve once the offending agent is withdrawn but mortality may be severe and prolonged recovery ensues. The overall mortality rate for drug_ induced liver injury is approximately 5% (Marc and George 1997). Therefore this research aimed at investigating possible ameliorative effect of aqueous extract of *zest of Citrus sinensis* (AEZCs) on cadmium chloride induced liver toxicity in wistar rats

MATERIALS AND METHODS

Plant collection and identification

The fresh fruits of *C. sinensis* were collected from a cultivated farm at Agbani, Enugu State, Nigeria. The zests of these fruits were obtained using a zester. They were identified and authenticated at the hebarium Unit of the Department of Crop Science University of Nigeria, Nsukka.

Chemicals, reagents and equipment

Chemicals/ reagents such as sodium hydroxide, potassium dihydrogen phosphate, hydrogen peroxide, trichloroacetic acid, hematoxylin, Eosin and sodium nitrate, hydrochloric acid etc. produced by Shijiazhuang Xinlongwei Chemicals .Ltd, Hebel China were purchased from Ogbete Main Market, Enugu, Nigeria They were of analytical grades of the highest purity. Standard laboratory equipment was used for this study. The sample bottles and kits for biochemical assay were purchased from Sigma Aldrich USA.

Preparation of the powdered zest of citrus sinensis

A total of 1000 oranges were peeled with a zester or grater while the white portion of the peel under the mesocarp was carefully avoided by limiting the peeling depth (Akunna et al., 2018). The zest was thoroughly rinsed with distilled water, dried at room temperature (27± 2°C) for 4 weeks and then reduced to powdered form by blending with a mechanical blender (Binatone Nigeria Ltd)

Preparation of the aqueous zest extract of citrus sinensis

400 g of the powdered sample of zest of *C. sinensis* was weighed and macerated in 1000 ml of distilled water and the mixture was thoroughly stirred after 8 h using a sterile glass rod. They were allowed to stand for 30 min before filtration using a muslin cloth. The filtrate was centrifuged at 3000 rpm for 10 min and the supernatant collected. The supernatant obtained was further cleaned off particles by suction using Whatman No 1 filter and cellulose paper. The extract was subsequently concentrated to dryness in vacuum at 40°C using a rotary evaporator (LE -10105)

Table 1. Phytochemical analysis of AEZCS.

Phytochemicals	*Citrus sinesis*
Alkaloids	++
Tannins	+
Phenols	+
Saponins	++
Flavonoids	+++

(+)= Mildly present, (++)= Moderately present, (+++)=Highly present.

and stored in desiccators. Different fresh solutions of the extract were prepared in normal saline as vehicle.

Phytochemical analysis

The phytochemical analysis of the constituents present in the AEZCS was done according to the method of Rizk 1982 using Wagner's reagents.

Alkaloids

The aqueous extract of the peel was separately evaporated to dryness and the residue heated on a boiling water bath (Marshal Scientific isotherm 220) with 2N HCl (5 ml). After cooling, the mixture was filtered and the filtrate divided into two equal portions. One portion of each mixture was treated with a few drops of Mayer's reagent and the other with equal amounts of Wagner's reagent (Rizk, 1982). The samples were observed for the presence of turbidity or precipitation. A letter sign probably (S+) score was used to record if the reagent produced only a slight opaqueness; (E+) score, if the definite turbidity, but no flocculation was observed and (E+++) score, if a definite heavy precipitate or flocculation was produced (Surmaghi et al., 1992).

Flavonoids

According to the method described by Somolenski et al. (1972), the presence of flavonoid was confirmed when pink or magenta–red color developed within 3 min of treatment of 5 ml each of the extracts with a few drops of concentrated HCl and magnesium turnings.

Saponins

2.5 g each of the plant extract was further extracted with boiling water. After cooling, the extract was shaken thoroughly to froth and then allowed to stand for about 15-20 min. The saponin content classification using the method described by Kapoor et al., (1969) was followed:

No froth=negative
Froth less than 1 cm =weakly positive
Froth 1:2 cm high =positive
Froth greater than 2 cm high= strongly positive

Tannins

Each of the extract was further extracted by 10 ml of 0.9% NaCl solution; it was filtered and divided into 3 equal portions. Sodium chloride solution was then added to one portion of each of the extracts, 1% gelatin solution to a second portion and the gelatin salt reagent to a third portion. Precipitation with a latter reagent or with both the second and third reagent was used in indication of the presence of tannins. Positive test was confirmed by the addition of FeCl3 solution to the extract which gave a characteristic blue, blue–black, green or blue green color and precipitate (Segelman and Farnsworth, 1969).

Method of acute toxicity test (LD_{50})

The acute toxicity test (LD_{50}) was determined according to the method of Enegide et al. (2013). In this method, wistar rats were procured, acclimatized and were administered different doses of the AEZCS. The administration was done in phases ranging from phase 1 to a maximum phase 4. Mortality or morbidity was monitored every 2 h for 10 min and monitoring in each phase lasted for a maximum of 24 h. When there was no mortality, the experiment proceeded to the next phase as explained in Table 1. Later, the maximal dosage where no mortality occurred and the minimal dosage that caused mortality were summed up and divided by 2 and their square root determined in accordance with Enegide et al. (2013).

Experimental animals

40 adult wistar rats purchased from the breeding stock from Animal House Unit of the College of Medicine, Enugu State University of Science and Technology, Parklane Enugu, were used for this research work. The animals were housed in standard rat's cages with proper ventilation at 12 h light/dark cycle. They were allowed to acclimatize for 14 days under standard natural photoperiodic condition with access to food and water *ad libitium.* All experimental procedure involving the animal care were conducted in conformity with International, National and Institutional guidelines for the care and use of Laboratory animals in biomedical research as promulgated by the Canadian Council of Animal Care (CCAC, 1985) and the guideline principles for research in Helsinki Declaration of 1979 was adhered to.

Experimental design

A total of 40 adult wistar rats were randomly divided into eight groups of five rats each as stated below.
Group A: received 5 mg/kg of $CdCl_2$ without treatment
Group B: received 10 mg/kg AEZCs orally for 8 weeks
Group C: received 40 mg/kg AEZCs orally by gastric lavage
Group D: received 5mg/kg $CdCl_2$ as single dose for 2 weeks+ 10 mg/kg AEZCS daily for 6 weeks
Group E: received 5mg/kg $CdCl_2$ as single dose daily for 2 weeks + 40 mg/kg AEZCS for 6 weeks
Group F: received 10 mg/kg AEZCS daily for 2 weeks + 5 mg/kg

$CdCl_2$ as single dose, 24 h after treatment with AEZCS
Group G: received 40 mg/kg AEZCS daily for 2 weeks + 5 mg/kg Cadmium chloride as single dose 24 h after treatment with AEZCS
Group H: received 10ml/kg normal saline for 8 weeks

Animal sacrifice and sample collection for analysis

The animals were observed daily for signs of toxicity during the experimental period. At the end of the treatment period, all animals were fasted for 12 h before sacrifice and organ harvest. The abdominal cavity was opened up following a midline abdominal incision made to expose the organ of study for harvest. Following its harvest, it was then washed thrice in ice cold saline and blotted on ash free paper for macroscopic inspection before being weighed with an electronic analytical and precision balance (Metler Nigeria ltd). The weight of the liver of each animal was taken. The estimation of the liver to body weight ratio was determined by comparing the weight of each organ with the final body weight of each rat as described by Ashafa et al. (2011)

Collection of blood sample

Blood samples were collected by cardiac puncture method under 25% Urethane anesthesia with the aid of a 5 ml hypodermic syringe (Hindustan syringes and Medical Devices Ltd, Faridabad, India). The blood samples were collected into tubes containing 2% sodium oxalate and centrifuged at 3000 rpm for 10 min using a table top centrifuge (P/C 03) (Model No. HR20, Zhengou, Henan China) and serum extracted. Sera were separated using cooling centrifugation and stored in aliquots at –25°C for biochemical assays of specific liver enzymes.

Method of determination of alkaline phosphatase activity

This was done by optimized standard method recommended by the Geseiischage fur Klinishche Chemic GSCC (1972).P –Nitrophenyl phosphate is hydrolyzed to phosphate and p– nitrophenol in the presence of ALP. A calculated amount of sample 0.01 ml in a test tube was mixed with the reagent (0. 5 ml) containing the substrate p nitrophenyl phosphate and brought to room temperature. The solution was mixed, initial absorbance read after 1 minute. The reaction was then allowed to stand for 3 min and the absorbance read against 405 nm. The enzyme activity was calculated thus:

U/L= 3300 x Absorbance of the test sample at 405 nm/min MACRO

Measurement of alanine and aspartate aminotransferases (ALT and ALP)

The measurement of AST and ALT activities in the serum was done using end point colorimetric diagnostic kits (Randox; laboratories UK) based on the method of Ofem et al. (2014). The pyruvate produced by transmission reaction between L–alanine and ketoglutarate reacts with 2,4- dinitrophenyl hydrazine to give a colored hydrazone which represents alanine aminotransferease activity. The oxaloacetate hydrazine formed with 2, 4–dinitrophenyl hydrazine is used to measure aspartate aminotransferease (AST). Both ALT and AST were read at 540 nm wavelength. The enzyme activity was obtained from the table after plotting a graph of absorbance against enzyme activity as provided in the leaflet/ manual.

Measurement of total serum protein

A widely used method of measuring serum total protein is the biuret reaction. The principle of this reaction is that serum protein reacts with copper sulphate in sodium hydroxide to form violet “biuret complex”. The intensity of the violet color is proportional to the concentration of the proteins

Determination of the biochemical parameters of the oxidative stress

Preparation of the tissue homogenates for the biochemical assays

The liver specimen were weighed and homogenized separately with potter-Elvenhjem homogenizer. The liver tissue was homogenized in potassium phosphate buffer 10 Nm pH (7.2) for estimation of MDA level and SOD activity. The crude tissue homogenate was centrifuged at 10,000 rpm for 15 min in a centrifuge and the resultant supernatant was used for the different estimation using the Analyzer Gold Kits.

Determination of Malondialdehyde (MDA) level in tissues

The level of the tissue MDA was determined using the method of Ohkawa et al. (1979). Thus, 0.2 ml of the supernatant homogenate was pipetted out followed by addition of 0.2 ml of 8.1% sodium dodecyl sulphate, 1.5 ml of 30% acetic acid (pH 3. 5) and 1.5 ml of 30% thiobabituric acid. The volume was made up to 4 ml with distilled water. The test tube was incubated for one hour at 95^{o}C and then cooled. 1ml of distilled water was added followed by the addition of 5 ml of n– butanol pyridine mixture (15: 1 w/v). The tubes were centrifuged at 1000 rev per minute for 10 min. The absorbance of the developed pink color was measured spectrophotomically (DU 640B spectrophotometer) Beckman Coulter, Inc CA USA at 532 nm.

Determination of superoxide dismutase level in tissues

Tissue SOD enzymes activity was assayed utilizing the technique of Ekaterina and Bernard (2004). The total superoxide activity was determined at 500 nm by measuring the inhibition of xantine-xanthanic oxidase mediated reduction of 2-3-bis(2-methoxy-4-nitro-5-sulfophenyl-2H-tetrazolidium -5-carboxamide (XTT); 0.5Mm xanthine oxidase sufficient to produce a slope of 0.25,50µM XTT, 25°C. A single unit of the enzyme was expressed as 50% inhibition of nitrobluetetrazolium (NBT) reduction/min/protein.

Tissue processing

The right lobe of the liver was excised and fixed in 10% formaldehyde solution. Subsequently, it was dehydrated in increasing concentrations of alcohol (80-100% v/v). The tissue was then cleared in xylene, embedded in paraffin blocks and sectioned at 5 µm thickness on a Leica Rotary Microtome. The tissues were stained with hematoxylin/eosin (H & E). Photomicrographs of the liver tissue sections were then taken after evaluation of the tissue of the tissue histology under light microscope. The tissue architecture were assessed for nuclear variations, loss of normal architecture of the parenchymatous tissue, cytoplasmic vascularization in both peripartal and central areas, cellular degeneration and necrosis, fat globules formation and lipid infiltrations.

Table 2. Acute toxicity of AZECS in wistar rats.

Phases	Treatments (mg/kg)	Number of deaths
ONE	10	0/8
	100	0/8
TWO	1000	0/4
	1500	0/4
THREE	3000	0/4
	5000	4/4

LD_{50} OF AEZCS = $\sqrt{3000 x 5000}$ =3873 mg/kg.

Table 3. Animal body weights, liver weights and liver/body weight ratio (g).

Groups	Initial body wt	Final body wt	Liver weight (g)	Liver/body wt ratio
A	318.40±16.35	327.80±39.78	8.47±1.22	0.03
B	297.05±33.98	307.77±27.24	8.02±0.27	0.03
C	257.97±28.1	186. 60±14.77	6.25±4.25	0.03
D	180.42±18.24	231.50±22.11	10.65±1.57	0.05[a]
E	165.47±10.72	215.10±13.52	8.57±0.38	0.04[b]
F	180.47±12.67	221.10±31.16	8.32±1.87	0.04[b]
G	251.07±45.90	194.40±14.38	6.97±4.69	0.04[b]
H	275.57±21.04	201. 65±13.25	12.75±0.50	0.06*

*Represents significant difference at P<0.05 when compared with the negative control (Group H); [ab] represents significant difference at P<0.05 when compared with the positive control. Those without superscript show that there was no significant difference (P>0.05). Values were expressed as Mean ±SD, n=5.

Statistical analysis

Data obtained were analyzed using a Statistical Package for Social Sciences (SPSS Version 21) and were expressed as Mean ± standard error of mean (SEM) using One way Analysis of Variance (ANOVA) with Tukey Post–hoc test. P<0.05 was considered statistical significant difference

RESULTS

As Shown in Table 1, flavonoids had the highest amount in the extract of the zest of *C. sinesis* while tannins and phenols had the least. Alkaloids and saponins were present in moderate amounts. Table 2 shows that at varying doses of AEZCS there were no mortality or signs associated with oral toxicity except at the maximum dose of 3780 mg/kg body weight of AEZCS which resulted in a toxicity signs; significant loss of fur skin and lesions, diarrhea, salivation, tremors, coma and eventually death of the rats.

Table 3 shows that there was a significant increase (P>0.05) in the liver/body weight (LW/BW) ratios following the administration of cadmium chloride in the positive control group of rats. Similar increase was also noted in the liver and body weight ratio in the rat groups post treated with low dose of AEZCs after cadmium exposure. In contrast, the liver or body weight ratios in the rat group pretreated with the low dose of AEZCs was significantly lower than observed in the positive control group. Significant decreases in the liver /body weight ratios was also observed in the group of rats that were pretreated and post treated with the high dose of AZECS.

As shown in Table 4, the concentration of Malondialdehyde and superoxide dismutase activity in the positive control (Group A) was significantly higher (P>0.05) than the test groups. There was significant increase in the concentration of malondialdehyde of the groups of rats pre-treated and post-treated with the low doses of AEZCs (groups F and D respectively).The increase in the groups pre-treated and post –treated with AEZCS was accompanied by an increase and decrease in the activity of superoxide dismutase respectively. In contrast, there were significant decrease (P>0.05) in the concentration of malondialdehyde in the groups pre-treated and post-treated with the high dose of AEZCS (groups G and E) with a concomitant surge and decrease in the activity of superoxide dismutase respectively. The variations in the activity of lipid peroxidation in the tissue.

Table 5 shows the concentration of the serum liver enzymes. The ALT was more pronounced in groups A (83.80±8.00)u/L, followed by group F (73.24±2.95), Group D (72.90±6.02) u/l etc with the least value observed in group H (47.85±3.26). Group A has the maximum concentration of ALP level followed by groups

Table 4. Concentrations of Malondialdehyde and Superoxide dismutase on aqueous extract of zest of *Citrus sinensis* on cadmium chloride induced liver toxicity.

Groups	SOD (U/mg protein)	MDA (nmol/mg protein)
A	4.17±0.42	8. 5±1.01
B	22.62±2.14	1.01±0.17
C	18.8±0.33	1.15±0.26
D	24.83±0.99	3.77±0.05
E	20.83±1.93	3.76±0.41
F	26.30±2.78	2.48±0.55
G	30.65±1.33	4.77±0.08
H	19.89±0.36	1.81±0.06

Values were expressed Mean ±SD, n=5. Those without superscript shows that there was no significant difference (P>0.05) .

Table 5. Serum liver enzymes activity of aqueous extract of zest of *Citrus sinensis* on cadmium chloride induced liver toxicity.

Groups	ALT (U/L)	ALP (U/L)	AST (U/L)	Total protein (g/100 ml)
A	83.80±8.06*	537.05±36.26*	66.71±3.39*	6.36±0.62*
B	51.17±4.34	226.90±4.63	29. 51±1.69	8.12±0.44
C	60.11±11.32	215.86±6.44	29.2±1. 56	7.74±0. 60
D	72.90±6.02 [a]	496.28±9.65 [a]	52.43±0.95[a]	7.12±0.01 [a]
E	62.63±9.14 [b]	373.87±8.92 [b]	45.27±2.49 [b]	6.95±0.21 [a]
F	73.24±2.95 [a]	420.31±42.28 [a]	61.03±4.27 [a]	7.28±0.18
G	60.30±7.11 [b]	309.66±31.46 [b]	40. 53±3.30[b]	6.88±1.00[a]
H	47.85±3.26	246.69±0.72	33.79±1.81	6.46±0.43

* represents significant increase or decrease at P<0.05 when compared with the negative control (Group H); [ab] represents significant difference at P<0.05 when compared with the positive control.

D, F with values of 537.05±36.26, 496.28±9.65 and 420.31±42.28 u/l respectively. The least values were in groups C (215.86±6.44) u/L. Meanwhile similar pattern of their concentration was observed in AST concentration with group A, F, D and G having the least values respectively. In their total protein concentration, group B has the highest values followed by groups C and F with group A having the least concentration.

Histological results

Photomicrographs of the histological sections of the control groups of wistar rats were compared with those of the treatment groups. Histological changes were observed in the peri-portal hepatocytes, sinusoidal arrangement, portal area etc. These changes were used to assess the depth of hepatic damage and recovery following treatment with AEZCS as well as to substantiate evidences obtained in the biochemical analysis (Figures 1 to 8).

DISCUSSION

In the acute toxicity test, the animals did not show any sign of aggression or unusual behavior during handling throughout the 14 days observational period following oral administration of the different doses of the aqueous extract of the zest C*. sinensis*. The extract did not produce any mortality up to 3783 mg/kg body weight and this is not in keeping with similar finding of LD_{50} of citrus peel extract by Saalu et al. (2006). It is therefore suggestive that the zest extract of *C. sinensis* may not be toxic to health at the administered dose. Phytochemical analysis of AEZCS showed a highest percentage abundance of flavonoids. This finding is in keeping with the report by Lu and Foo (2004) that flavonoids are potent free radical scavenger and super anti-oxidant conferring anti-peroxidative properties in most plant extracts. The extract of zest citrus sinensis also showed increased proportions of alkaloids and saponins and this may also have contributed to the medicinal benefits of AEZCS. This is in agreement with the submission by

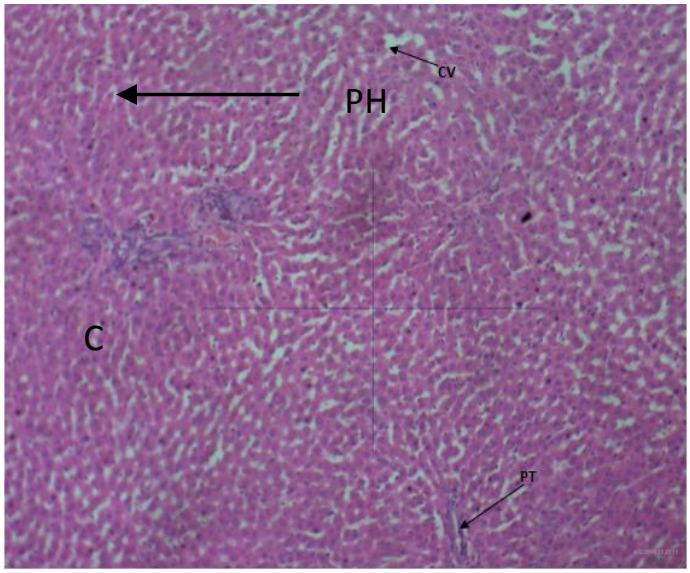

Figure 1. Photomicrograph of rat liver in positive control (Group A) shows hepatic cells without necrotic zones (NZ) and prominent halos (PH).Central Vein (CV). H & E stain x400.

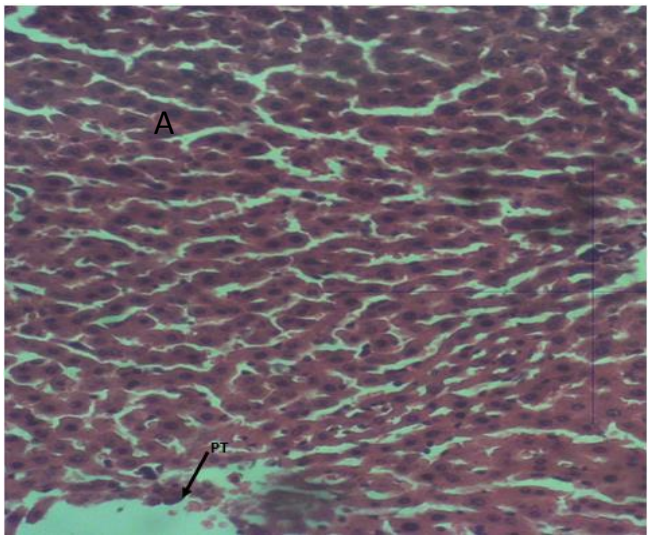

Figure 2. Photomicrograph of rat liver treated with low dose of AZECs alone (group B) Portal Area (PA), Hepatic plates (HP),vein (CV) with hepatic sinusoids. HE stain x400.

Milugo et al. (2013) that alkaloids and saponins contributes to health; being actively involved in body metabolism and development. Generally, our finding on the presence and varying proportions of phytochemical in AZECS is in line with that of Srividhya et al. (2013). The toxicological experiment, comparison of organ/ body weights between treated and untreated groups of animals have conventionally been used to evaluate toxicity in target organs (Peters and Boyd, 1966; Pfeiffer, 1968). In this study, the increase in the liver/body weight ratios following the administration of cadmium chloride in the positive control group of rats may be attributed to tissue swelling. This finding on the organ-body weight ratio is in keeping with Amresh et al. (2008) that toxic chemicals may contribute to organ swelling, atrophy or hypertrophy. Generally, our finding showed that of hepatocytic damage

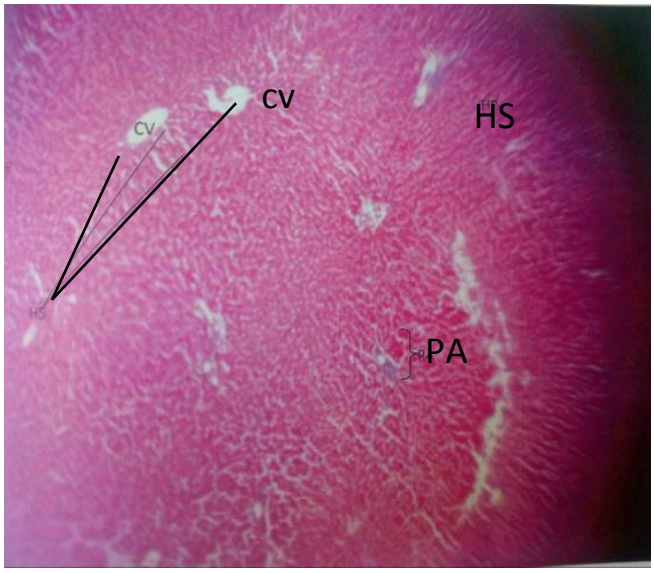

Figure 3. Photomicrograph of rat liver treated with low dose of AZECs alone (group C) showing preserved Central vein (CV) with hepatic sinusoids (HS), Portal Area Plates (HP),.HE stain x400.

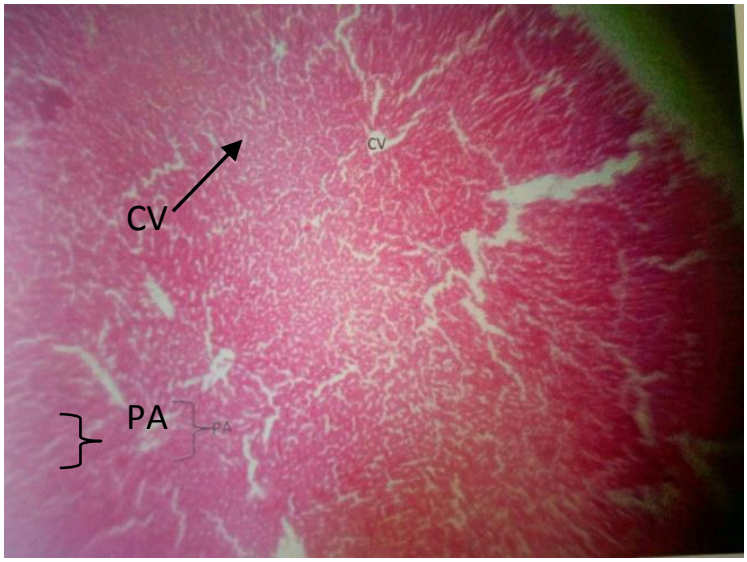

Figure 4. Photomicrograph of rat liver treated with cadmium chloride and shortly followed with low dose AZECS (Group D) showing Central vein (CV) and, Hepatic prominent Halos (PH).H & E stain x400.

relative to the animal body weight was minimal since the liver/body weight ratios in the groups treated with AEZCS before and after exposure to cadmium did not significantly differ from that of the negative control. Using the index, our finding on the ameliorative and protective benefit of AEZCs is in line with the work of Udoh et al. (2005) on the seed extract of *C. papaya.* When the liver was exposed to cadmium chloride, there were nodular deposits of whitish brown substances believed to be adipose tissues of the liver. The positive control groups of rats shed light on the potential impact of chronic exposures to environmental toxicants like cadmium on the liver health and in particular on the presence of diffused portal area (PT) and sinusoidal dilations. These

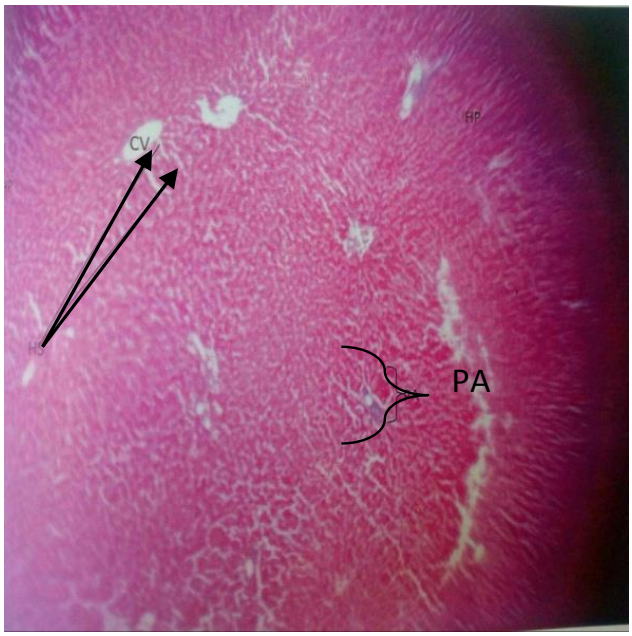

Figure 5. Photomicrograph of rat liver treated with Cadmium chloride and shortly followed with low dose of AZECs (Group E) shows distinct Portal area (PA), Central vein (CV)and Hepatic sinusoids which were otherwise distorted in the photomicrograph of the positive control. H & E stain x400.

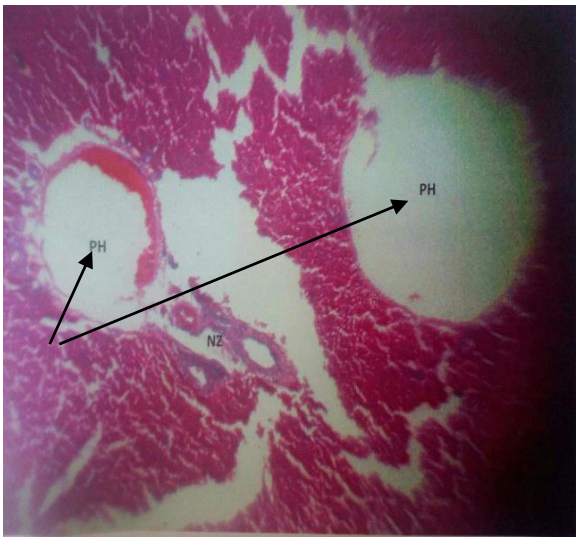

Figure 6. Photomicrograph of rat liver treated with low dose of AZECS before Cadmium chloride administration (Group F) showing Central vein (CV) and prominent Halos (PH) H & E stain x400.

toxic contaminants have a great steatogenic potential and needs to be considered tangible as risk factors as a result of cadmium chloride toxicity. Suzuki et al. (2014) reported that the factors that mediate occurrence of liver disease are oxidative stress, tissue hypoxia, and immune response and membrane alterations. This present study collaborates the report by Omar et al. (2013) that prolonged exposure to critical level of cadmium is

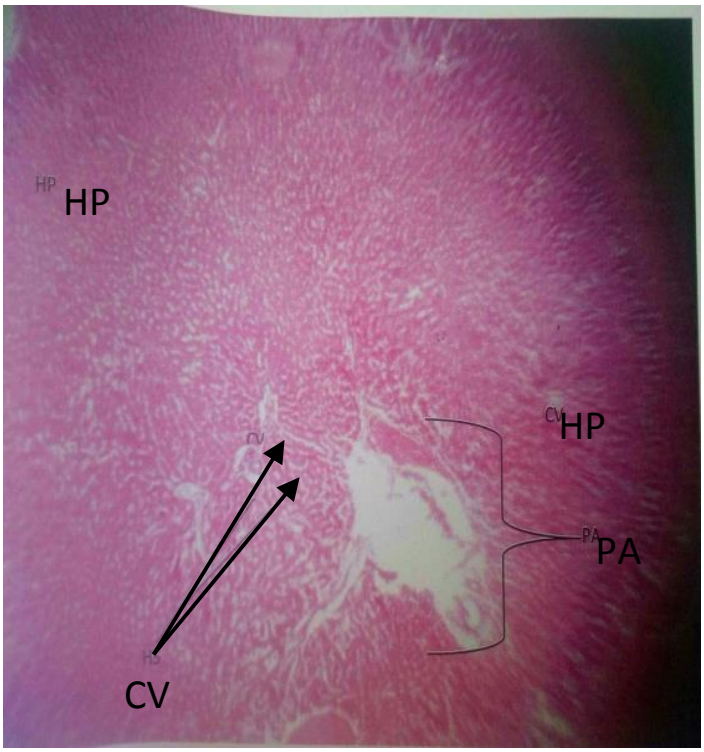

Figure 7. Photomicrograph of rat liver treated with low dose of AZECS before Cadmium chloride administration (Group G) shows Hepatic plates (HP), Central vein (CV and radiating hepatic sinusoids (HS) and Area (PA) which are in contrast to the photomicrograph of the positive control but similar to that of the negative control. H & E stain x400.

associated with incidences of liver diseases. The effect of AEZCS on the histological appearance of the liver shows tortuous, dilated hepatic sinusoids lined by a discontinuous layer of fenestrated endothelial cells that also exhibit fenestration and discontinuous basal lamina. The structure and the tortuous path of hepatic sinusoids through the liver bed allow for an efficient exchange of materials between the hepatocytic and blood. According to Yamano et al. (2000), hepatic endothelial cells might be the first cellular target for cadmium induced hepatocellular injury. Our findings on the histopathological changes associated with cadmium chloride exposure are in consonance with the report of El-Sokkary et al. (2010)

There was extensive destruction of fenestrations on the luminal surface of endothelial cells which supports the findings of Kuester et al. (2002). This condition is marked by the extrusion of damaged endothelial into the capillary lumen, producing local ischemia and subsequent activation of Kupffer cells as well as polymorphonuclear neutrophils (PMN) infiltration. These events trigger a cascade of inflammatory mediators that promote necrosis. These cellular changes may have also resulted in apoptosis since studies in rats, mouse or human hepatocytes show that apoptosis play a role in cadmium hepatotoxicity (Lasfer et al., 2008; Yu et al., 2011).

The liver histology of the rats pretreated with doses of 10 and 40 mg/kg prior to the exposure to cadmium revealed significant differences in their liver histology. However, only high dose of AEZCS showed better histoarchitectural preservation of the parenchyma. Our work supports the findings of Saalu et al. (2006) on the protective effect of high dose of 10 and 40 mg/kg after exposure to cadmium revealed significant attenuating changes in the liver histology. However, this ameliorative change in the liver parenchyma was better observed at the high dose of 40 mg/kg. Our work supports histological studies on dose dependent ameliorative effect of the Citrus peel extract (Parmar and Kar, 2008; Saalu et al., 2006).

Biochemical changes of the liver in our positive control group of rats which received a single dose of cadmium chloride without treatment with AZECS revealed excessive lipid peroxidation with an increase in superoxide dismutase (SOD) activities. This finding is in line with the report by Watkins (1992) that excessive lipid peroxidation using MDA index and an increase in superoxide dismutase (SOD) activities are hallmark of oxidative stress. These biomarkers of oxidative stress

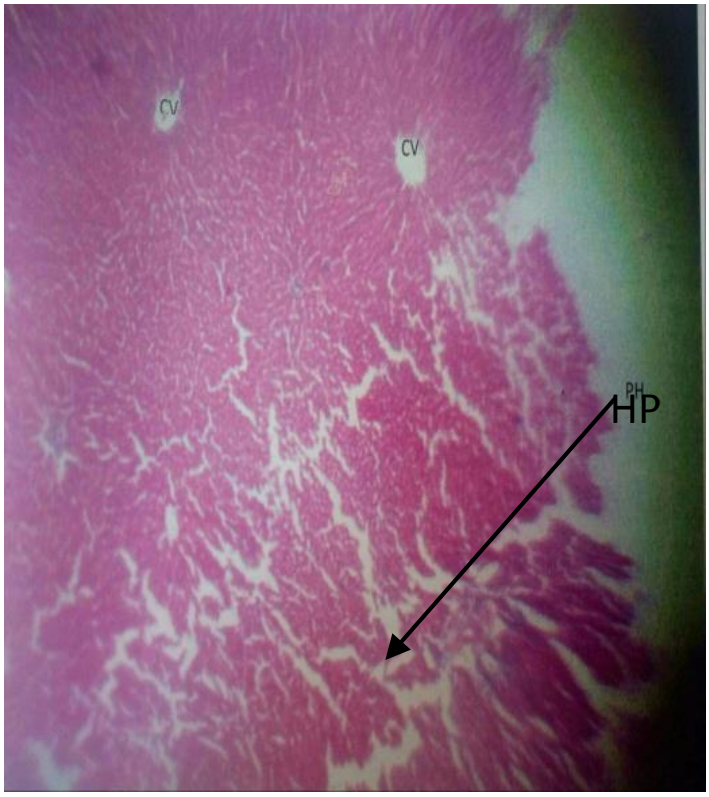

Figure 8. Photomicrograph of rat liver in negative control group showing normal hepatic plates (HP) with well preserved cytoplasm and nucleus, Portal Area (PA), Hepatic sinusoids (HS), Portal radiating with Central vein (CV),towards the periphery. H & E stain x400.

were also observed in the rats pretreated with low dose and high dose of AEZCS before cadmium exposures. Sustained increase in SOD and MDA levels suggested that AEZCS may be unable to protect the hepatocyte from oxidative damage at low doses; the biochemical role of AEZCS to better protect the liver against cadmium toxicity may be associated with the high dose. This supports report on protective efficacy of high dose of AEZCS against testicular damage (Saalu et al., 2006).

Following the post-treatment of cadmium toxicity with low and high dose of AEZCS, biochemical evidence showed an ameliorative change in SOD activity and MDA level. Our findings showed a decrease in MDA concentrations after treatment of toxicity with the high dose of 40 mg/kg of AEZCS being more effective. This supports similar report by Nada et al. (2014) on the ameliorative effect of peel of citrus sinensis against castration induced oxidative stress on the liver.

Hence, the effect of the AEZCS on the liver enzymes and total protein concentration showed that the liver injury induced through cadmium chloride administration culminated in increased concentrations of Alanine aminotransferease (ALT), Aspartate aminotransferease (AST) and alkaline amino phosphatase (ALP). The rise in levels of these enzymes particularly ALT, is in concordance with report in hepatocellular membrane damage (Ahmadizadeh et al., 2013). AEZCS administration at both lower and higher doses protected and maintained the liver function by moderating the activities of serum liver enzymes. Although this potency was more efficient at the high dose AEZCS at our higher dose, significantly lowered the serum liver enzymes activities which is increasingly altered in oxidative stress conditions as reported by Friedman et al. (1996). Thus, the ameliorative potential of AEZCS supports the findings by Kaplan (1993) that a decline in liver enzymes activity after exposure to toxic agents usually indicates recovery. Though, this may not be a strong prognostic sign in fulminant liver injury, where there are major losses of functional hepatocyte. In the positive control group of rats, cadmium exposure significantly decreased the level of serum total proteins. The abnormal decrease has been associated to damage in the hepatic sinusoids and blood vessels in oxidative stress condition (Friedman et al., 1996). A consequence of decrease in serum albumin being the major plasma protein is a shift of blood from the intravascular to interstitial spaces and peritoneal cavity resulting in intravascular volume depletion and edema

formation (Busher, 1990). However, in the groups of pretreated and post treated with low and high doses of AEZCS before and after exposure to cadmium, there was a noticeable increment in the total protein suggesting its protective and ameliorative potency against cadmium induced serum protein depletion

Conclusion

Hepatotoxicity from most environmental toxicants has posed a predominant health risk in various populations especially in sub-Saharan Africa. The treatment options are few, usually expensive, less accessible and not synthetic treatment products since the extract of zest of *C. sinensis* contains important phytochemical constituents. From the results obtained in the research, the extract of the zest of *citrus sinensis* significantly protected and ameliorated liver associated with cadmium in our study environment. Hence, this research recommends that attempt should be made to insert the extract as residues in dietary supplements and in drug formulation for treatment of liver dysfunction associated with cadmium.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors are seriously acknowledged including the laboratory technologists who carried out the experimental aspect of the research.

REFERENCES

Akunna GS, Obikili EN, Anyanwu GE, Esom E (2018). Histochemical and Morphometric evidence of the curative role of aqueous extract of citrus sinensis on anti-neoplastic drug induced testicular degeneration of animal model. European Journal of Anatomy 22(6):497-507.

Ahmadizadeh M, Esmailpoor M, Goodarzi Z (2013). Effect of Phenobarbital on chloramphenicol induced toxicity in rat's liver and small intestine. Iranian Journal of Basic Medical Sciences 16(12):1282-1285.

Amresh GR, Singh PN, Rao VC (2008). Toxicological screening of traditional medicine Laghupatha (Cissampelos pareira) in experimental animals. Journal of Ethnopharmacology 116:454-460.

Answar AS, Ahmad S, Ulleh R, Nasar MA, Fouad H, Rehman N, Hussain H and Wajid S (2014).Phytochemical and Biological activities of four wild medicinal plants. Scientific World Journal. 2014:857363.

Ashafa OT, Sunmonu TO, Afolayan AJ (2011). Effect of leaf and berry extracts of *phytolacca dioica* L. on hematological and weight parameter of wistar rats. African Journal of Pharmacy and Pharmacology 5(2):150-154.

Agency for Toxic Substances and Disease Registry (ATSDR) (1989). Toxicological profile for cadmium. ATSDR/U.S. Public Health Services, Atlanta, ATSDR/TP-88/08.

Busher JT (!990) . Serum Albumin and Globilin . Clinical Methods Chapter 10. 3rd Edition.

Ekaterina VZ, Bernard R (2004). Effect of glutathione depletion on antioxidants enzymes in the epidiymis, seminal vesicles, liver and spermatozoa motility in the aging brown Norway rats. Biology of Reproduction 71(3):1002-1008

El-Sokkary GH, Nfady AA, Shabash EH (2010). Melatonin administration ameliorates cadmium induced oxidative stress and morphological changes in the liver of rat. Ecotoxicology and Environmental Safety 73:456-463.

Enegide C, Arome D, Solomon AF (2013). A new method of determining acute toxicity in animal models. Toxicology International 20(3):224-226.

Etebu E, Nwauzoma AB (2014). A review on sweet orange (*Citrus sinensis* Osbeck): health, diseases and management. American Journal of Research Communication 292:33-70.

Friedman LS, Martin P, Munoz SJ (1996). Liver function test and the objective of evaluation of the patient with liver disease. In: Zakkin D and Boyer TD, eds. Hepatology; a textbook of liver disease, 3rd edn. Philadelphia: WB Saunders pp. 791-833

Geeta RA, Kalidhar SB (2010). Biological activities of Citrus sinensis varieties. A review. Agricultural Research Centre. Agricultural Reviews 31(4):267-278

Goyer R (1991). Toxic effects of metals. In: Amdur, MO., Doull J.D and Klassen CD. Eds. Casarett and Doull's Toxicology.4th edn. Pergamon Press New York pp. 623-680.

Kaplan MM (1993). Laboratory tests. In: Schiff ER, eds. Diseases of the liver, 7th ed. Philadelpha: JB Lippincott pp. 108-144.

Kapoor LD, Singh A, Kapoor SL, Srinivastava SN (1969). Survey of Indian plants for saponins, alkaloids and flavonoids. LIoydia 32:297-304.

Kuester RK, Waalkes MP, Goering PL, Fisher BL, McCuskey RS (2002). Differential Hepatotoxicity induced by cadmium in Fischer 344 and Sprague –Dawley rats. Toxicology Science 65:151-159.

Lasfer M, Vadrot N, Aoudjehane L, Conti F, Bringuier AF (2008). Cadmium induced mitochondria-dependent apoptosis of normal human hepatocytes. Cell Biology and Toxicology 24:55-62

Lu L, Foo LY (2001). Antioxidant activity of polyphenols from sage (Salvina Officinalis). Food Chemistry 75:197-202.

Marc GS, George HL (1997). Xenobiotics- induced hepatotoxicity: mechanism of liver injury and methods of monitoring hepatic function. Clinical Chemistry 43 (8):1512-1526.

Milugo TK, Omosa LK, Ochanda JO, Owuor BO, Wamumyokoli FA, Oyuji JO, Ochibong JW (2013). Antagonistic effects of alkaloids ad saponins on bioactivity in the quinine tree (rauwolfia caffra sond): Further evidence to support biotechnology in traditional medicinal plants. BMC Complementary and Alternative Medicine 13:285.

Nada AEM, Amany AT, Elgamal B, Abdelmonein AE (2014). Ameliorative effect of citrus peel extract and castration induced oxidative stress in liver and kidney of rats. Journal of Applied Pharmaceutical Science 4(107):64-68.

Nicolosi E, Deng ZN, Gentile A, La Malfa S, Continella G,Tribulato E (2000). Citrus phylogeny and genetic origin of important species as investigated by molecular markers. Theoretical and Applied Genetics 100 (8):1155-1166.

Ofem EO, Nna VU, Archibong NA (2014). Comparative effects of two antimalarial drugs (P– alaxin and coartem) on serum electrolytes and serum enzymes in albino wistar rats. Research Journal of Pharmaceutical, Biological and Chemical Sciences 5(1):54–63.

Ohkawa H, Ohishi N, Yagi K (1979). Assay for lipid peroxidation in Animal tissues by thiobarbituric acid reaction. Analytical Biochemistry 95(2):351-358.

Omar H, Michael C, David C, Joseph MH, Li AF e,Firoozmand AG, Ayman K, Timothy MP (2013). Cadmium exposure and liver disease among US Adults. Journal of Gastrointestinal Surgery 17(17):1265–1273.

Parmar HS, Kar A (2008). Medicinal values of fruits peel from citrus sinesis, Punica granatum and Musa Paradisiacal with respect to alterations in tissue lipid peroxidation and serum concentration of glucose, insulin and thyroid hormones. Journal of Medicinal Food 11(2):376-381.

Peters JM, Boyd EM (1966). Organ weights and water levels of the rats following reduced food intake. Journal of Nutrition 90(4):354-360.

Pfeiffer CJ (1968). A mathematical evaluation of the rhythmic weight parameter. Toxicology and Applied Pharmacology 13(2):220-227.
Piccinelli AL, Mesa MG, Armenteros DM, Alfonso MA, Arevalo AC, Campone L, Raetrelli L (2008). HPLC–PDA–MS and NMR characterization of C–Glycosyl flavones in a Hydroalcoholic extract of citrus aurantifolia leaves with antiplatelet activity. Journal of Agricultural and Food Chemistry 56:1574-1581.
Rizk AM (1982).Constituents of plants growing in Qatar: A chemical survey of sixty plants. Filoterapia 52:35-44.
Saalu LC, Osinubi AA, Jewo PL, Oyewopo AO, Ajayi GO (2006). An evaluation of influence of citrus paradise seed extract on doxorubicin induced testicular oxidative stress and impaired spermatogenesis. Asian Journal of Scientific Research 3:51-61.
Segelman AB, Fanworth NR (1969). Biochemical and phytochemical screening of plants IV. A new rapid procedure for the simultaneous determination of saponins and tannins. LIoydia 32:59-65.
Somolenski SJ, Silins H, Farnsworth NR (1972). Alkaloid screening I'. LIoydia 35:1.34.
Srividhya M, Ramanathan K and Krishnanand N (2013). Efficacy of citrus fruits peel extract against pathogens causing gastrointestinal disorders. International Journal of Pharmacy and Pharmaceutical Sciences 5(4):160-163.
Surmaghi MHS, Aynehchi Y, Amin GH, Mahmoodi Z (1992). Survey of Iranian plants for saponins, alkaloids, flavonoids and tannins, IV. DARU Journal of Pharmaceutical Sciences, pp. 281-291
Suzuki T, Shinjo S, Arai T, Kanai M, Goda N (2014). Hypoxia and fatty liver. World Journal of Gastroenterology 20(41):15087-15091.
Udoh VF, Udoh PU (2005). Hepatotoxicity of the methanol extract of carica papaya (paw paw) seeds in wistar rats. Journal of Pharmaceutical Biology 34(4):349-352.
Watkins PB (1992). Drug metabolism by cytochrome P450 in the liver and small bowel. Gastroenterology Clinics of North America 21:511-526.
Shin HJ, Park KK, Lee BH, Moon CK, Lee MO, (2003). Identification of genes that are induced after cadmium exposure by suppression suntractive hybridization. Toxicology 191 (2-3): 121-131.
Favela-Hernández JM, González-Santiago O, Ramírez-Cabrera MA, Esquivel-Ferriño PC, Camacho-Corona Mdel R (2016). Chemistry and Pharmacology of citrus sinensis. Molecules 2 (2): 247.

Vol. 14(1), pp. 18-26, January-March 2020
DOI: 10.5897/AJBR2019.1058
Article Number: B43B85763340
ISSN 1996-0778
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African Journal of Biochemistry Research

Full Length Research Paper

Lambda-Cyhalothrin induced hepato-nephro toxicity potentials and post treatment recovery in *Clarias garipinus*

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Received 8 October, 2019; Accepted 17 March, 2020

This evaluates the 28-day toxicity and 7-day post treatment effect of LCT on the behaviour, liver and kidney of *Clarias gariepinus.* Prior to the experiment, fishes were acclimatized for two weeks. 120 fishes of standard length (SL) / weight (W) 10-12 cm, 8 - 17 g were used for median lethal concentration (LC_{50}) test and 120 fishes of SL / W 16 - 40 cm, 200 - 250 g were used for the behavioural, hepato - nephrotoxicity and 7-day post treatment tests. The behavioural response of *C. gariepinus* upon exposure to LCT was observed from 24 to 96 h. The experiment had four treatments with LCT concentrations of 0.00, 2.5 x 10^{-4} µg/L, 5.0 x 10^{-4} µg/L and 6.25 x 10^{-4} µg/L and 30 fishes per treatment in triplicates for 28 days. In days 1, 7, 14, 21 and 28 of treatment and 7 days after treatment, fishes were brought out for blood samples collected through caudal alteration for liver and kidney marker enzymes tests (alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), creatinine and urea) using standard methods. There was a concentration dependent increase in faster swimming movement, hyperactivity, jerky movement, gulping of air, repeated closing and opening of the mouth and percentage mortality of *C. gariepinus* exposed to LCT. ALT, AST, ALP, creatinine (CR) and urea levels showed concentration and duration significant increased ($p < 0.05$) while total protein significantly decreased ($p < 0.05$) compared with controls. After 7 days of depuration, ALT, AST, CR and total protein were not different from the control. This study has demonstrated that LCT caused hepato-nephrotoxicity in *C. gariepinus.* The severity of LCT hepato-nephro in *C. gariepinus toxicity* was evident in this studies because ALP and urea levels did not return to normal after 7 days of depuration.

Key words: Liver enzymes, kidney enzymes, toxicity, lambda-cyhalothrin,behavioural responses, *Clarias gariepinus.*

INTRODUCTION

Agrochemicals especially pesticides are indirectly consumed by human via food chain. Man's pursuit to meet up with the increase in food demand has led to various technologies and production of synthetic chemical used for eliminating superfluous pests and scheming disease vectors. Consequently, there are notable cases of adverse environmental effects on the non-targeted organisms mainly in aquatic ecosystem

(Murthy et al., 2013).

Lambda cyhalothrin (LCT) is a synthetic pyrethroid (an insecticide) used for the eradication of several insects at home and agricultural fields (Mergel, 2000). The LCT is normally routed into the soil through discharge of remains of materials used for packaging and storage and accidental discharge during spraying, then the aquatic ecosystem through runoff resulting from its use in agricultural field (De Moraes et al., 2013). The major risk of synthetic pesticide is environmental contamination especially the natural water systems where it causes various deleterious effect in aquatic resources (fish) and ultimately in human. Researches have proven that fresh water fish diversity is threatened by a number of environmental stressors such as contaminants and nutrient loading, habitat degradation and climate change (Jelks et al., 2008). Accumulation of synthetic pesticides results in huge number of residues in the environment, thereby causing a substantial hazard in the environment due to its uptake and accumulation in the food chain and drinking water (Somdare, 2015). Edward et al. (2013) reported that though toxic chemicals in water may be below detectable levels when sampled, but the concentrations due to bioaccumulation found in examined fish parts were beyond tolerable levels. Ginebreda et al. (2014) established that organisms in aquatic environments are exposed to a complex mixture of chemicals including parent compounds and their transformation products which cause multiple damages in the organisms, population and ecosystem level due to effect on organ function, reproductive stages and biological diversity.

Based on the above background, the present study evaluated lambda-cyhalothrin induced hepato-nephro toxicity potentials and post treatment recovery in *Clarias gariepinus.*

MATERIALS AND METHODS

Collection of experimental fish

A total of 240 *C. gariepinus* (with standard length and weight that ranged from 16 to 40 cm and 80 to 250 g respectively) procured from Freedom Fisheries Ltd, University Market Road, Nsukka, Enugu State, Nigeria, was used for this study. They were transported to the Laboratory in aerated bags. The fish were disinfected in 0.05% potassium permanganate ($KMnO_4$) solution for two minutes to avoid dermal infections and later acclimatized for two weeks in plastic tanks of 300 L capacity. They were fed daily with food (Coppens commercial feed) containing 40% crude protein. The fecal matter and other waste materials were siphoned off and water changed daily to reduce ammonia content in the water during experimentation. Dead fish was removed with forceps to avoid possible deterioration of the water quality. During acclimatization the water was changed after 48 h with well aerated tap water.

Procurement of the test compound

A commercial formulation of lambda-cyhalothrin (600 gl^{-1}), batch number 160227 marked by Amanik Agro Investment Limited Lagos, Nigeria, was purchased at Ogige Local Market Nsukka, Enugu state, Nigeria.

Physico-chemical parameters of the test water

Some physico-chemical parameters (temperature, dissolved oxygen, pH, nitrate and nitrite) of the test water were analysed following the protocol of APHA (1992).

Determination of median lethal concentration (LC_{50})

Prior to experiment, determinations of the $LC_{24\text{-}96\ h}$ of LCT were conducted using 120 fish. Triplicate sets of 10 fish were randomly exposed to LCT at concentrations of 0.0, 5.0 x 10^{-4}, 6.25 x 10^{-4}, 7.5 x 10^{-4} and 8.75 x 10^{-4} µg/L derived from a range finding test using plastic tanks of 40 L capacity each. Ten L of water was poured into each tank. Another set of 10 fish (replicated three times) was simultaneously maintained with equal amount of tap water but without the test compound and considered as control. Fish were not fed throughout the experiment and toxicity of the toxicity end point was observed. Fish was physically examined daily and considered dead in the absence of respiratory movement and swimming in response to gentle touch. Dead fish were removed and mortality was recorded at intervals of 24, 48, 72 and 96 h. The $LC_{24\text{-}96}$ values of the insecticide for the species at24, 48, 72 and 96 h were determined by Probit analysis (Finney, 1971).

Determination of safe levels

The safe levels of the test compound were estimated by multiplying the 96 h LC_{50} with different application factors (AF) and were based on the methods of (Hart et al., 1948; Sprague, 1971; CWQC, 1972; NAS/NAE, 1973; IJC, 1977; CCREM, 1991).

Experimental design for sublethal exposure

The experiment consisted of four treatments of 0.00, 2.5 x 10^{-4} µg/L, 5.0 x 10^{-4} µg/L and 6.25 x 10^{-4} µg/L (A-D) in replicate. Each tank contained 10 L dechlorinated tap water with 10 fishes in each of the tank. The exposure period was 28 days during which the fish were fed with small quantity of feed approximately 1% of total body weight an hour before the test solution was renewed daily. On each sampling day, (7, 14, 21 and 28), three to five fish from each of the treatment group including the control were sacrificed after anesthetizing with tricaine methanessulfonate (MS 222) to minimize stress. Blood samples were collected (through caudal alteration)

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for liver and kidney marker enzymes test. After the end of the sublethal exposure, the fish in each of the concentrations were withdrawn from the exposure of the chemical and were placed in chemical-free water after which further observation was made after 7 days of the withdrawal.

Behavioural responses

Some behavioural responses (hyperactivity, swimming patterns, fin movement, buccal cavity and gills) of *C. gariepinus* upon exposure to different concentrations of LCT were observed from 24 h to 96 h of the exposure.

Determination of liver marker and kidney marker enzymes

Liver marker enzymes AST, ALP and ALT levels were determined using the standard method described by (Reitman and Frankel, 1957). The total protein and albumin levels were determined using the Biuret method as described by (Sood, 2006). Determination of kidney markers enzymes - blood urea (BUN) and creatinine (CR) levels were determined according to the method of (Bartels and Bohmer, 1972).

Statistical analysis

Data was analysed using Statistical Packages for Social Sciences (SPSS) 20.0 (IBM Corp, Armonk, USA) and Statplus v 5.9.8 (AnalystSoft Inc., Walnut, Canada). Probit regression analysis using the Finney Method (lognormal distribution) was for lethal concentration (LC). Two-way Analysis of Variance (ANOVA) was used to compare concentration of LCT and duration of exposure dependent effects. The means were separated using DMRT (Duncan Multiple Range Test). Level of significance was set at $p < 0.05$.

RESULTS

Physico-chemical parameters of the water used for the experiment

The physico-chemical parameters of the water used for the experiment at different concentration level of LCT are shown in Table 1. The pH level and dissolved oxygen of test water after exposure to different concentrations of LCT showed no difference from each other. The pH level and dissolved oxygen observed were all concentration-dependent as indicated in Table 1 while NO_2 and NO_3 were not present in the water used for the experiment as the values recorded were 0.0 both in control and treatment groups.

Behavioural responses of *C. gariepinus* exposed to lambda-cyhalothrin

Behavioural responses of *C. gariepinus* exposed to LCT at different concentration levels for 96 h are presented in Table 2. In group A (control), from 24 - 96 h of exposure, no mortality and behavioural changes were observed as fish exhibited normal swimming patterns, normal body and fin movements. The treatment groups B, C, D and E displayed varied behavioural abnormalities as the concentration increased. Faster swimming movement, hyperactivity, jerky movement, rapid fin and opercula movement, gulping of air, repeated closing and opening of the mouth were more severe in groups D and E which led to loss of balance and finally death (Table 2).

Median lethal concentration (LC_{50})

Cumulative mortality of fish exposed to different concentration levels of Lambda cyhalothrin

Percentage mortality of *C. gariepinus* exposed to graded concentrations of LCT at 24 h increased with increase in toxicant concentration Table 3. Fishes exposed to 6.5 x 10^{-4} µg/L, 7.5 x 10^{-4} µg/L and 8.75 x 10^{-4} µg/L had 3.3%, 23.3% and 20.0% mortality respectively while no death was recorded in 5.0 x 10^{-4} µg/L and the control. The percentage mortality at 48 h did not follow concentration gradient.

The highest mortality at the 96 h was recorded in the group D exposed to 7.5 x 10^{-4} µg/L toxicant concentration with 66.7% death when compared to other concentration. No absolute (100%) mortality was observed at the end of the exposure.

Lethal concentration of Lambda-cyhalothrin (95% CI) × 10^{-4} µg/L depending on exposure time for C. gariepinus

The concentration at 5% lethality (LC_{50}) to LC_{99} of LCT for 24, 48, 72- and 96-h exposure of *C. gariepinus* is presented in Table 4. The LC_{50} exposure of *C. gariepinus* to LCT at 24 h exposure gave 10.7234 x 10^{-4} µg/L (95% CI, 0.00091364 – 0.00211) while LC_{50} at 48 and 72 h LCT was 9.8482 x 10^{-4} µg/L (95% CI, 0.00043134 – 0.0022485) and 8.2218 x 10^{-4} µg/L (95% CI, 0.00050983 – 0.0013259) respectively. Finally, the value at 96 h exposure to the highest graded concentration concentration of LCT was 8.163 x 10^{-4} µg/L (95% CI, 0.00049513 - 0.0013458). The toxicant concentration in all the groups exposed to LCT decreased as time progressed. LC_{99} of LCT at 24 h was 23.0092 x 10^{-4} µg/L (95% CI, 0.0014729 - 0.018895) and at 96 h was 13.8741 x 10^{-4} µg/L (95% CI, 0.00024477 - 0.00786417).

Estimation of safe level for *C. gariepinus* after 96 h exposure

The safe levels of LCT were obtained using different

Table 1. **T**he physico-chemical parameters of the experimental water exposed to different concentration levels of Lambda-cyhalothrin .

S/N	Treatment (µg/L)	Temperature (°C)	pH	DO (mg/l)	NO_2 (mg/l)	NO_3 (mg/l)
1	Control	23.11	6	5.8	0	0
2	5.0×10^{-4}	23.11	6.8	5.71	0	0
3	6.25×10^{-4}	22.12	6.6	5.55	0	0
4	7.5×10^{-4}	22.12	5.7	5.35	0	0
5	8.75×10^{-4}	22.12	5.7	5.35	0	0

DO = Dissolved oxygen; NO_2 = Nitrite; NO_3 = Nitrate.

Table 2. Behavioural responses of *C. gariepinus* exposed to Lambda-cyhalothrin at different concentration levels.

Concentration (µg/L)	Swimming rate	Fin movements	Hyperactivities	Jerky movement	Equilibrium status
24 h					
Control	+++	+++	-	-	+++
5.0×10^{-4}	+++	+++	-	-	+++
6.25×10^{-4}	+++	+++	-	-	+++
7.5×10^{-4}	++	++	-	-	++
8.75×10^{-4}	+	+	-	-	+
48 h					
Control	+++	+++	-	-	+++
5.0×10^{-4}	+++	+++	-	-	+++
6.25×10^{-4}	+++	+++	-	-	+++
7.5×10^{-4}	++	++	-	-	++
8.75×10^{-4}	+	+	-	-	+
72 h					
Control	+++	+++	-	-	+++
5.0×10^{-4}	++	++	-	-	++
6.25×10^{-4}	+	+	-	-	+
7.5×10^{-4}	-	-	+++	+++	-
8.75×10^{-4}	-	-	+++	+++	-
96 h					
Control	+++	+++	-	-	+++
5.0×10^{-4}	+	+	-	-	+
6.25×10^{-4}	+	+	-	-	+
7.5×10^{-4}	-	-	+++	+++	-
8.75×10^{-4}	-	-	+++	+++	-

None = -; Mild = +; Moderate = ++; Strong = +++.

Table 3. Mortality of *Clarias gariepinus* exposed to different concentrations of Lambda-cyhalothrin.

Group	Concentration (µg/L)	Treatment size	Mortality (%)			
		(n - 30)	24 h	48 h	72 h	96 h
A	0	30	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
B	5.0×10^{-4}	30	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
C	6.25×10^{-4}	30	1 (3.3)	1 (3.3)	1 (3.3)	1 (3.3)
D	7.5×10^{-4}	30	7 (23.3)	11 (36.7)	19 (63.3)	20 (66.7)
E	8.75×10^{-4}	30	6 (20.0)	7 (23.3)	13 (43.3)	13 (43.3)

Table 4. Lethal concentration of Lambda-cyhalothrin.

Percentile	Concentration (95% CI) x 10^{-4}µg/L			
	24 h	48 h	72 h	96 h
5	6.25(4.16 - 7.02)	5.90(2.75 - 12.65)	5.65(2.49 - 12.79)	5.61(2.35 - 13.41)
10	7.04(5.66 - 7.84)	6.61(3.83 - 11.38)	6.14(3.23 - 11.66)	6.09(3.07 - 12.11)
20	8.14(7.31 - 10.05)	7.58(4.94 - 11.64)	6.79(4.23 - 10.88)	6.74(4.06 - 11.17)
25	8.60(7.74 - 11.50)	8.00(5.06 - 12.61)	7.05(4.58 - 10.85)	7.00(4.42 - 11.10)
30	9.03(8.07 - 13.09)	8.37(5.01 - 13.96)	7.30(4.84 - 11.00)	7.24(4.68 - 11.21)
40	9.87(8.63 - 16.71)	9.10(4.71 - 17.59)	7.76(5.10 - 11.81)	7.71(4.95 - 11.99)
50	10.72(9.143 - 21.10)	9.85(4.31 - 22.49)	8.22(5.10 - 13.26)	8.16(4.95 - 13.46)
60	11.65(9.65 - 26.71)	10.65(3.89 - 29.16)	8.71(4.93 - 15.39)	8.65(4.78 - 15.66)
70	12.74(10.21 - 34.44)	11.59(3.46 - 38.85)	9.26(4.65 - 18.45)	9.20(4.49 - 18.86)
75	13.38(10.54 - 39.66)	12.15(3.23 - 45.66)	9.59(4.48 - 23.20)	9.52(4.30 - 21.05)
80	14.13(10.91 - 46.42)	12.80(2.99 - 54.73)	9.96(4.28 - 23.20)	9.89(4.09 - 23.88)
90	16.33 (11.93 - 70.32)	14.67(2.43 - 88.59)	11.01(3.75 - 32.35)	10.93(3.55 - 33.68)
95	18.40(12.84 - 99.15)	16.43(2.04 - 132.28)	11.96(3.34 - 42.86)	11.88(3.13 - 45.08)
99	23.01(14.73 - 188.95)	20.31(1.46 - 281.74)	13.97(2.66 - 73.31)	13.87(2.45 - 78.64)

CI = confidence interval.

Table 5. Estimated safe level of Lambda-cyhalothrin for *C. gariepinus* after 96 h.

Chemical	96h LC_{50} (µg/L)	Method	Application factor	Safe level (µg/L)
Lambda-cyhalothrin	8.163×10^{-4}	Hart et al. (1948) *	-	3.50291×10^{-7}
		Sprague (1971)	0.1	8.163×10^{-5}
		CWQC (1972)	0.01	8.163×10^{-6}
		NAS/NAE (1973)	0.01 – 0.00001	8.163×10^{-5} – 8.163×10^{-9}
		CCREM (1991)	0.05	4.0815×10^{-5}
		IJC (1977)	5% of 96h LC_{50}	4.0815×10^{-5}

application factors as indicated in Table 5. The calculated safe levels of LCT ranged between 8.163×10^{-5} and 8.163×10^{-9} µg/L.

Effects of Lambda-cyhalothrin on biomarkers of hepatotoxicity

The biomarkers of hepatotoxicity followed a distinct trend dependent on concentration of LCT and exposure duration. The ALT, AST and ALP levels increased significantly ($p < 0.05$) on day 28 compared to day 1 in groups exposed to the three concentrations of LCT ($p < 0.05$); in the control, there was no significant increase in these enzymes at the same duration ($p > 0.05$). ALT and AST in groups exposed to the concentrations of LCT normalised to baseline level at the end of 7-day recovery period (Table 6). The ALP level dropped significantly in groups exposed to the three concentrations of LCT at the end of 7-day recovery period compared to its level at the end of 28 days exposure, but baseline level was not attained. The AST and ALP levels at baseline were not different (at $p < 0.05$) between all the groups (control, 2.5×10^{-4}, 5.0×10^{-4} or 6.25×10^{-4} µg/L). The ALT level of all the groups exposed to LCT was not different ($p > 0.05$) from control, except 6.25×10^{-4} µg/L group which was less at $p < 0.05$. But from day 7, 14, and 21, ALT, AST and ALP concentration respectively were higher ($p < 0.05$) than control till on day 28.

Effects of Lambda-cyhalothrin on total protein

Total protein concentration was reduced with increase in concentration of LCT and duration of exposure (Figure 1). Total protein level in all the groups exposed to concentrations of LCT was significantly less than the control from day 14 to day 28 ($p < 0.05$). At the end of 7 days recovery period, total protein in all groups exposed to LCT was not different from the control.

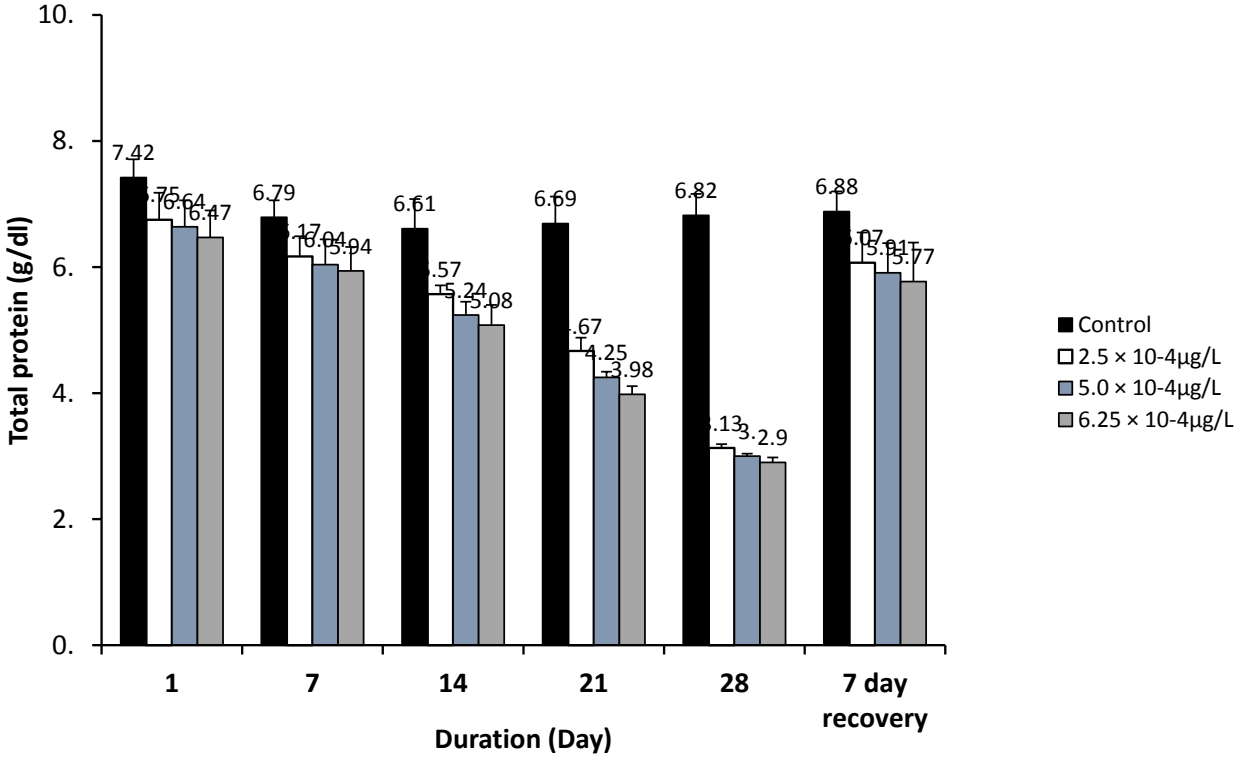

Figure 1. Variations in total protein on exposure of *Clarias gariepinus* to lambda-cyhalothrin.

Bars with different alphabet label for each group (control, 2.5 x 10^{-4}, 5.0 x 10^{-4} and 6.25 x 10^{-4}µg/L) were significantly between weeks of treatment ($p < 0.05$). Bars with different numeric superscript for each week were significantly different between treatment concentrations (control, 2.5 x 10^{-4}, 5.0 x 10^{-4} or 6.25 x 10^{-4}µg/L) ($p < 0.05$).

Effects of Lambda-cyhalothrin on biomarkers of nephrotoxicity

There was concentration and duration of exposure dependent effect of LCT on creatinine and urea concentrations. Both biomarkers of nephrotoxicity increased ($p < 0.05$) on day 28 compared to baseline level in 2.5 x 10^{-4}, 5.0 x 10^{-4} and 6.25 x 10^{-4}µg/L LCT exposed groups ($p < 0.05$); CR and urea were significantly higher than baseline from day 21 and day 14 respectively in the treatment groups (Table 7). Concentration of CR in control had some variations which was not significant within the same duration; but urea increased significantly on day 28 compared to days 21, 14, 7 and 1 value ($p < 0.05$). Seven days post-exposure to the concentrations of LCT, CR and urea levels reduced significantly ($p < 0.05$) compared to day 28 exposure levels ($p < 0.05$).

DISCUSSION

Variations in physicochemical parameters in water bodies' especially surface water bodies are indicative of the influence of anthropogenic activities (Nnamonu et al., 2018a). Physicochemical analyses serve as a sensitive tool for assessing the portability and vulnerability of water sourced for drinking and other domestic purposes (Nnamonu et al., 2018a). The temperature recorded in all groups was within the optimal range for fish production. This is in consonant with Keremi et al. (2014). Potential of hydrogen (pH) is a logarithmic scale for expressing the acidity or alkalinity of a solution. In water, it affects metabolism and physiological processes of fish and also exerts considerable influence on toxicity of ammonia. The pH observed (5.7- 6.0) was within desirable range and agreed with ICAR (2006). The DO values (5.35- 5.80 mg/L) recorded in this study align with Edward et al. (2013). Nitrite ($NO_{2)}$ and nitrate (NO_3) are introduced into the water bodies through run off waters. They are also introduced into ponds through dead phytoplankton, uneaten feeds, dead and decaying organic matter Keremi et al., 2014). The findings were in agreement and within

Table 6. Variations in Mean ± SE of selected biomarkers of hepatotoxicity on exposure of *Clarias gariepinus* to lambda-cyhalothrin.

Parameter	Concentration (µg/L)	Duration (Day)					7-day recovery
		1	7	14	21	28	
ALT (U/L)	Control	11.88 ± 0.06^{a2}	11.85 ± 0.08^{a2}	11.87 ± 0.07^{a1}	11.61 ± 0.32^{a1}	11.60 ± 0.32^{a1}	11.48 ± 0.24^{a1}
	2.5×10^{-4}	11.87 ± 0.07^{c2}	11.65 ± 0.10^{c12}	12.80 ± 0.29^{b2}	13.74 ± 0.48^{a2}	13.99 ± 0.02^{a2}	11.93 ± 0.04^{c1}
	5.0×10^{-4}	11.85 ± 0.03^{c2}	11.57 ± 0.11^{c12}	13.20 ± 0.32^{b2}	13.60 ± 0.20^{b2}	14.24 ± 0.07^{a2}	11.82 ± 0.07^{c1}
	6.25×10^{-4}	11.53 ± 0.16^{c1}	11.43 ± 0.16^{c1}	13.35 ± 0.29^{b2}	13.78 ± 0.17^{ab2}	14.29 ± 0.13^{a2}	11.76 ± 0.07^{c1}
AST (U/L)	Control	11.61 ± 0.21^{a1}	11.39 ± 0.44^{a1}	11.79 ± 0.18^{a1}	11.78 ± 0.18^{a1}	11.86 ± 0.07^{a1}	11.77 ± 0.12^{a1}
	2.5×10^{-4}	11.82 ± 0.13^{b1}	11.20 ± 0.42^{c1}	12.34 ± 0.10^{b12}	13.41 ± 0.11^{a2}	13.84 ± 0.14^{a2}	11.88 ± 0.05^{b1}
	5.0×10^{-4}	11.11 ± 0.58^{c1}	11.06 ± 0.46^{c1}	12.56 ± 0.24^{b12}	13.72 ± 0.17^{a2}	14.08 ± 0.12^{a2}	11.69 ± 0.10^{bc1}
	6.25×10^{-4}	10.81 ± 0.81^{b1}	10.91 ± 0.36^{b1}	13.00 ± 0.43^{a2}	13.87 ± 0.10^{a2}	14.16 ± 0.15^{a2}	11.48 ± 0.20^{b1}
ALP (U/L)	Control	49.62 ± 5.65^{a1}	51.00 ± 5.39^{a1}	49.61 ± 5.41^{a1}	50.06 ± 6.00^{a1}	50.28 ± 6.70^{a1}	50.30 ± 6.61^{a1}
	2.5×10^{-4}	48.83 ± 5.19^{c1}	65.83 ± 2.96^{b2}	85.22 ± 2.73^{a2}	89.31 ± 3.27^{a2}	93.83 ± 0.38^{a2}	61.49 ± 0.59^{b2}
	5.0×10^{-4}	49.50 ± 5.09^{c1}	66.56 ± 3.08^{b2}	87.00 ± 3.28^{a2}	89.83 ± 3.37^{a2}	94.52 ± 0.10^{a2}	62.69 ± 0.53^{b2}
	6.25×10^{-4}	50.83 ± 4.04^{c1}	67.89 ± 2.99^{b2}	87.44 ± 2.73^{a2}	93.56 ± 5.18^{a2}	95.03 ± 0.17^{a2}	63.00 ± 0.93^{b2}

Values with different alphabet superscript across a row were significantly different; and values with different numeric superscript down a column were significantly different ($p < 0.05$). ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; ALP, Alkaline phosphatase.

Table 7. Variations in selected biomarkers of nephrotoxicity on exposure of *C. gariepinus* to lambda-cyhalothrin.

Parameter	Concentration (µg/L)	Duration (Day)					
		1	7	14	21	28	7-day recovery
CR (mg/dl)	Control	0.52 ± 0.08^{a1}	0.59 ± 0.08^{a1}	0.52 ± 0.08^{a1}	0.52 ± 0.08^{a1}	0.44 ± 0.13^{a1}	0.47 ± 0.03^{a1}
	2.5×10^{-4}	0.82 ± 0.07^{c2}	0.59 ± 0.08^{cd1}	0.74 ± 0.07^{cd2}	1.24 ± 0.07^{b2}	1.62 ± 0.06^{a2}	0.52 ± 0.08^{d1}
	5.0×10^{-4}	0.82 ± 0.07^{c2}	0.74 ± 0.07^{c1}	0.89 ± 0.00^{c23}	1.42 ± 0.07^{b23}	1.84 ± 0.06^{a23}	0.67 ± 0.13^{c12}
	6.25×10^{-4}	0.89 ± 0.00^{c2}	0.82 ± 0.07^{c1}	0.96 ± 0.07^{c3}	1.59 ± 0.10^{b3}	2.00 ± 0.13^{a3}	0.82 ± 0.07^{c2}
Urea (mg/dl)	Control	24.17 ± 0.90^{b1}	24.60 ± 0.89^{b1}	25.71 ± 1.02^{b1}	24.04 ± 1.16^{b1}	37.29 ± 2.13^{a1}	36.92 ± 1.70^{a1}
	2.5×10^{-4}	26.14 ± 1.13^{c12}	26.70 ± 1.66^{c1}	36.31 ± 4.13^{b2}	51.38 ± 1.34^{a2}	54.49 ± 0.66^{a2}	39.39 ± 0.62^{b12}
	5.0×10^{-4}	26.87 ± 0.89^{c12}	27.51 ± 1.94^{c1}	40.21 ± 2.11^{b2}	52.59 ± 1.61^{a2}	56.92 ± 0.96^{a2}	41.23 ± 0.49^{b2}
	6.25×10^{-4}	27.73 ± 1.09^{c2}	29.06 ± 1.96^{c1}	41.48 ± 1.92^{b2}	53.34 ± 1.67^{a2}	57.62 ± 1.14^{a2}	42.22 ± 0.55^{b2}

Values as mean ± S.E. For each parameter, values with different alphabet superscript across a row were significantly different; and values with different numeric superscript down a column were significantly different ($p < 0.05$).

desirable range of 0.0 to 10.00 as reported by WHO (2010). The behavioural alteration and loss of equilibrium exhibited by the *C. griepinus* exposed to different levels of LCT is an indication that the region of the brain which is associated with the maintenance of (equilibrium must have been affected by LCT exposure. The supports Odo et al. (2016). This would result in prolonged neuromuscular depolarisation, culminating in the observed uncoordinated and jerky movement that was noticed in *C. griepinus* exposed to LCT (Sarai et al., 2013). We also observed repeated opening and closing of the mouth and operculum covering accompanied by partially extended fin. These behavioural changes are caused by hypoxic conditions which hampers oxygen uptake in fish. Hypoxic conditions arise primarily due to damage of gills of fish exposed to insecticides. These reports were consistent with (Somdare, 2015).

There was increased mucus secretion by the experimental animals which could be an adaptive response to counter the irritating effects of the insecticides on body surface and mucus membrane. This is in agreement with the report of Odo et al. (2016). The observed behavioural changes as demonstrated in our study might have affected swimming behaviour, feeding activities, predation, competition and reproduction.

This study has demonstrated that mortality of *C. gariepinus* exposed to LCT was concentration and exposure duration dependent. By implication, LCT is highly toxic to fish and other aquatic animals. This report is in line with Taofeek et al. (2013). Remarkably, the LC_{50} values of the present study decreased as the exposure time increased from 24 to 96 h due to effects of toxicant. The variation in the safe level as demonstrated in this study showed that differences obtained were all dependent on concentration and duration to LCT exposure.

The increase in AST, ALP and ALT agrees with Marzouk et al. (2012) while the increase in ALP disagrees with Bhushan et al. (2010). These enzymes are secreted in to blood in hepatocellular injury and their levels increase. The enhanced activities of transaminases ALP, AST and ALT revealed the hepatic damage / degeneration in LCT- treated group. These increases may be mainly due to the leakage of these enzymes from the liver cytosol into the bloodstream (Odo et al., 2016; Nnamonu et al., 2018b).

The increase of soluble liver enzymes in blood serum may be useful as an indicator of hepatic dysfunction and hepatocellular damage (Sloss, 2009). The significant increase in creatinine and urea agrees with Donadio et al. (1997). The increased plasma creatinine and urea levels reflect the diagnosis of renal failure (Donadio et al., 1997). Moreover, elevated blood urea is known to be correlated with an increased protein catabolism in mammals and/or the conversion of ammonia to urea as a result of increased synthesis of arginase enzyme involved in urea production. The significant increase ($p < 0.05$) in urea and creatinine levels depicts renal injury in the LCT-treated fish.

Conclusion

The high mortality rate of *Clarias gariepinus* exposed to LCT, significant elevations in liver and kidney marker enzymes confirm the severity of LCT toxicity. The severity of LCT hepato-nephro in C. toxicity in *gariepinus* is so evident in our studies because ALP and urea levels did not return to normal after 7 days of depuration.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

American Public Health Association, APHA (1992). Standard Methods for Examination of Water and waste 18th ed. American Public Health Association, Washing ton D.C

Bartels H, Bohmer M (1972). Clinical chemistry. Acta Tropica 37:193-98.

Bhushan B, Saxena N, Saxena PN (2010). Beta-cyfl uthrin induced histochemical alterations in the liver of albino rat. Scandinavian Journal of Laboratory and Animal Sciences 37:61-6.

CCREM - Canadian Council of Resources and Environmental Ministry (1991). Canadian Water Quality Guidelines, Inland Water Directorate Environment Canada: Ottawa, UN, Canada.

CWQC-Committee on Water Quality Criteria (1972). A Report of the Committee on Water Quality Criteria Ecological Research Series, EPA-R3-73-003, US Environmental Protection Agency Report; CWQC: Cincinnati, OH, USA.

De Moraes FD, Venturini FP, Cortella LRX, Rossi PA and Moraes G (2013). Acute toxicity of pyrethroid-based insecticides in the Neotropical freshwater fish *Bryconamazonicus".* Ecotoxicology and Environmental Contamination 8(2):59-64.

Donadio C, Lucchesi A, Tramonti G, Bianchi C (1997). Creatinine clearance predicted from body cells mass is a good indicator of renal function. Kidney International 52:166-168.

Edward JB, Idowu EO, Oso JA, Ibidapo OR (2013). Determination of heavy metal concentration in fish samples, sediments and water from Odo-Ayo River in Ado-Ekiti, Ekiti-State, Nigeria. International Journal of Environmental Monitoring and Analysis 1(1):27-33.

Finney YT (1971). Probit Analysis. London: Cambridge University press.

Ginebreda A, Kuzmanovic M, Guasch H, López de Alda M, López-Doval JC (2014). Assessment of multi-chemical pollution in aquatic ecosystems using toxic units: Compound prioritization, mixture characterization and relationships with biological descriptors. Science of Total Environment 468/469:715-723.

Hart WB, Weston RF, Dermann JG (1948). An apparatus for oxygenating test solution which fish are used as test animals for evaluating toxicity. Transaction of American Fisheries Society 75:288-236.

ICAR - Indian Council of Agricultural Research (2006). Current science recent development in agricultural research - agricultural science. Indian Agricultural Research Institute 90:1.

IJC-International Joint Commission (1977). New and Revised Great Lake Water Quality Objectives. Windsor: Ontario.

Jelks HL, Walsh SJ, Burkhead NM, Countreras-Balderas S, Diazpardo E, Hendrickson DA, Lyons J, Mandrak NE, McCormick F, Nelson JS,

Platania SP, Porter BA, Renaud CB, Schmitter-Soto JJ, Taylor EB, Warren ML (2008). Conservation status of imperiled North American freshwater and diadromous fishes. Fisheries 33:372-407.
Keremi RI, Davies OA, Abazi ID (2014). Physico-chemical analysis of fish pond water in freshwater areas of Bayelsa state, Nigeria. Greener Journal of Biological Sciences 4(2):033-038.
Marzouk MA, Abbassy MA, Mansour SA, Shaldam HA. (2012). Liver function alterations induced by lambda-cyhalothrin in male albino norway rats rattus norvegicus: ameliorative effect of zinc. Egyptian Journal of Agricultural Research 90(2):263-284.
Mergel M (2000). Lambda-Cyhalothrin. http://www.toxipedia.org/display/toxipedia/Lambda-Cyhalothrin. Accessed May 4th, 2019.
Murthy KS, Kiran BR and Venkateshwarlu M (2013). A review on toxicity of pesticides in fish. International Journal of Open Science Research 1:15-36.
NAS/NAE-National Academy of Sciences/National Academy of engineering (NAS/NAE) (1973). Water Quality criteria. EPA-R3-033; US Government printing Office: Washington, DC, USA.
Nnamonu EI, Ejere VC, Mbegbu EC, Ezechukwu CS, Ejim AO (2018b). Effects of methanolic calyx extract of *Hibiscus sabdariffa* on body weight, blood cholesterol and liver marker enzymes in Wistar rats. Journal of Medicinal Plants Research 12(26):427-434.
Nnamonu EI, Nkitnam EE, Ugwu FJ, Ejilibe OC, Ezenwosu SU, Ogbodo GU (2018a). Physicochemical Assessment of Vulnerability of the River Ebenyi in Eha-Amufu and Environs, Southeast Nigeria. Annual Research and Review in Biology 27(5):1-9.
Odo GE, Agwu JE, Madu J, Ezea CO, Nnamonu EI, Eneje VO (2016). Histopathological effects of Cyperdicot and vitamin E supplementation on selected organs of *Clarias gariepinus* (Burchell, 1822) reared in a tropical fish farm in Nigeria. African Journal of Biotechnology 15(9):303-314.
Reitman S, Frankel S (1957). Colorimetric method for the determination of serum oxaloacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology 28(1):56-63.
Sarai RS, Kopp SR, Coleman GT (2013). Acetylcholine receptor subunit and p-Glycoprotein transcription patterns in levamisole-suscesptible and resistant *Haemonchus contortus*. International Journal of Parasitology 3:51-58.
Sloss A (2009). Prescribing in liver disease. Australian Prescriber 32:32-35.
Somdare PO (2015). Oxidative Stress Biomarkers, Haematological Parameters and Histopathological Changes in the African Catfish, *Clarias Gariepinus* Exposed to an Organophosphate Pesticide, Fenthion: Unpublished Project Submitted to the Department Of Zoology and Environmental Biology, University of Nigeria, Nsukka.
Sood R (2006). Medical Laboratory Technology. Jaypee Brothers medical Publishers Limited. New Delhi. P 675.
Sprague JB (1971). Measurement of Pollutant toxicity to fish: biology assay method for acute toxicity. Water Resources, 3:793-821.
Taofeek AY, Olatunde OF, Adekunle AB (2013). Evaluation of toxic effects of Lambdacyhalothrin on the haematology and selected biochemical parameters of African catfish *clarias gariepinus*. Zoology and Ecology 10:1-8.
WHO-World Health Organization (2010). Environmental Health criteria: International programme on chemical safety: Geneva, Switzerland.

Vol. 14(1), pp. 27-32, January-March 2020
DOI: 10.5897/AJBR2019.1072
Article Number: 0028E7263342
ISSN 1996-0778
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African Journal of Biochemistry Research

Full Length Research Paper

Study of some biochemical parameters of the seeds of the fruit of the sweet Maniguette (*Aframomum alboviolaceum* (ridl.) k. Schum.) harvested in the Republic of the Congo

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Received 17 December, 2019; Accepted 17 March, 2020

***Aframomum alboviolaceum* is a plant that belongs to the family of Zingiberaceae, genus Aframomum and species of *A. alboviolaceum*. It is wide spread across tropical Africa and represented about 50 species. Proximate analysis is usually done to determine the values of macronutrients present in plant or food sample; it gives the composition of the biomass in terms of gross components. Proximate analysis includes determination of: Ash (sodium, potassium, iron, calcium, phosphorus) and other dietary minerals; moisture, proteins, fats, carbohydrate (dietary fibre, sugars, sugar alcohol, e.t.c). *A. alboviolaceum* (ridl.) k. schum) harvested in Republic of the Congo proximate analysis revealed the presence of lipids (10.58%); humidity (30.68%); protein (5.19%); carbohydrate (52.37% with 3.86% of dietary fibre); ash (1.18%) (Phosphorus=0.14%, Calcium=0.72%, Magnesium=0.29%, Iron=0.00%). The calculated energy value is 325.46 Kcal / 100 g.**

Key words: *Aframomum alboviolaceum* (Ridl.) K. Schum., fruit, seeds, physico-chemical.

INTRODUCTION

The Congo, like other countries of Central Africa has significant agricultural potential thanks to its climate, which unfortunately are insufficiently exploited and makes the country dependent on food imports. In recent years, there is a renewed interest in non-conventional crops with both potential assets for the development of populations at the local level as the industry (Silou et al., 2004). That is why seventy oil species in the basin of the Congo, from 35 botanical families were studied; their oil content and their fatty acid compositions were determined. Very numerous works have been published on this topic (Binaki et al., 2013; Kapseu, 2009; Loumouamou, 2012; Womeni et al., 2011; Attibayeba et al., 2010; Silou, 2014). Despite all this work of valorization of oilseeds in the Congo basin, much of our fruit seeds have never been subjected to scientific studies in this area.

The sweet *A. alboviolaceum* (Ridl.) K. Schum., of African origin, is known for its aromatic seeds fruit and plays an important role in native medicine or as spice or flavouring agents (Ngakegni, 2012). It is consumed fresh

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Figure 1. Fruit ripe integers *of A. alboviolaceum* (Ridl.) Lenaerts Schum.

and also used as raw material in the manufacture of juice first. The purpose of this study is to characterize the seeds of the sweet *A. alboviolaceum* (Ridl.) Lenaerts Schum.

This study is on the valorization of the seeds of *A. alboviolaceum* (Ridl.) Lenaerts Schum) and the determination of their nutritional potential.

MATERIALS AND METHODS

Plant material

The plant material of this study consists of seeds of the fruits of *A. alboviolaceum* (Ridl.) Lenaerts Schum, harvested in most of the savannas of the Republic of the Congo. Figures 1 to 4 show the fruits and seeds *of A. alboviolaceum* (Ridl.) Lenaerts Schum.

Methods

Determination of moisture (H)

The humidity level was determined according to the *AOAC* method (AOAC, 2005). 2 g of crushed obtained after grinding the seeds was placed in a previously weighed capsule and put in oven (Memmert, Germany) at 70°C until the mass became constant.

Determination of the rate of ash and major mineral

2g of crushed seeds meal was used for the determination of the rate of ashes by the gravimetric method (AOAC, 2005). Incineration of the samples was performed in an oven mitten at 550°C for 6 h. The rate of ash after incineration was calculated.

The mineral elements contents are measured by atomic absorption spectrophotometry (Perkin-Elmer-1100) on ash obtained after mineralization. Prior to dosing, the ashes are diluted in a solution containing 10% as corrector of interactions lanthanum chloride (concentration: 116 LaCl3 g in 1 l of HCl concentrated diluted to one-quarter).

Determination of the rate of fat (MG)

The lipids in 5 g of the dried and crushed seeds were extracted using Soxhlet (NF ISO 82 62 - 3, 2006) by 200 mL of hexane for 6 h. The excess of solvent is evaporated to the rotavapor (IKA HB 10 basic).

Determination of the rate of protein (P)

About 0.1 g of crushed seeds is used to determine the rate of the crude protein from the dosage of total by the method of Kjeldhal nitrogen (AOAC, 2005). Protein was obtained by multiplying the total nitrogen by a convention factor, 6.25.

Determination of the rate of total carbohydrate (G) and dietary fibre (FB)

Carbohydrate (G) was estimated by the difference method. According to this method (AOAC, 2005), it was calculated by subtracting the sum of moisture (H), of the fat (MG), protein (P) and (C) ash content in the sample of 100.

Raw samples fiber levels are determined by the method of Weende (Wolff, 1968). To do this, 1 g of the crushed seeds (M) is boiled in 50 ml of sulfuric acid (0.25 N) and then 50 ml of soda (0.31 N) for 1 h. The resulting residue is dried at 105°C for 8 h and then cremated at 550°C for 3 h.

Determination of the energy value (EV)

Total energy value was calculated according to the method of

Figure 2. Half- fruits of *A. alboviolaceum* (Ridl.) Lenaerts Schum showing the pulp and the seeds.

Figure 3. Dried seeds *of A. alboviolaceum* (Ridl.) Lenaerts Schum.

Manzi (1999) cited by Diallo et al. (2015)
It is determined using the formula below:

VE (kcal / 100g) = (CHO x 4) + (CL x 9) + (CP x 4) with CHO = % of carbohydrates.

Where, CL = % of lipids and CP = % protein.

RESULTS AND DISCUSSION

Moisture level

The different tests for the moisture content obtained gave an average of 30 68% on the seeds of *A. alboviolaceum* (Ridl.) K. Schum studied. This humidity is less than 56.33%, value obtained on fresh almonds *Borassus aethiopum* (Kabiru et al., 2015) and also very low compared to the *Cocos nucifera* (94.45%) (Jean et al., 2009). This allows us to conclude the fresh seeds *Aframomum alboviolaceum* (Ridl.) K. Schum studied are less hydrated than *C. nucifera* and therefore keeps a little better than the last.

So for better conservation, the seeds must be dried beforehand. This value is also high compared with those obtained by various authors on other products such as

Figure 4. Seeds dried and ground *of A. alboviolaceum* (Ridl.) Lenaerts Schum.

peanuts with: 7.48% (Ayoola and Adeyeye, 2010; Ayoola et al., 2012) on seeds (raw groundnut, sun-dried groundnut and roasted groundnut), 7.54% (Eshun et al., 2013) on the varieties sur les variétés Huitzuco 93, Rio Balsas, Ocozocuautla, Tlaxmalac Gerardo Uribe, Ranferi Diaz, A-18 and RF - 214 in Mexico; 5.55-6.05% (16) on the varieties *Sinkarzie, F - mix, JL 24, and Manipintar*; 4.12-4.75% (Mora-Escobedo et al., 2015; Brintha et al., 2014) on a variety of peanut in Sri Lanka after treatments of organic fertilizers; 7.18% (Adegoke et al., 2014) on a variety of peanut in Nigeria. It is however slightly less than those of fresh almonds of the Hyphaeneguineensis which is 37.32%. This water content is not normal for a good preservation of seeds (the conservation of seed water content ranges from 10 and 14%).

Rate of fats

The seeds of *A. alboviolaceum* Soxhlet extraction (Ridl.) K. Schum give an average fat content of 10.58%. This lipid content in seeds of the sweet *A. alboviolaceum* (Ridl.) K. Schum (10.58% is close to 8-10% on the same product (Ngakegni, 2012). On the other hand, this value is very low compared to that of the kernels of *C. nucifera*, which is 60% more (www.information nutritionnelle.fr) but very high compared to 0.01% value obtained from *B. aethiopum* (Kabiru et al., 2015). These seeds are poor in oil compared to walnuts *Juglans regia* L. (58.3-65.2%) (Tapia et al., 2013).This content is very low compared to 46, 10% (Ayoola et al., 2012), 40 to 42% (Mustapha et al., 2015); about 46% (Olayinka et al., 2015) and 39.30% (Adegoke et al., 2014). Some authors, by studying the physicochemical properties of eight varieties of peanuts grown in the Mexico, got the oil content in seeds ranging from 37.9 to 56.3% (Mora-Escobedo et al., 2015). This value of 10.58% is very low compared to 67.5% (Balla and Baragé, 2008) value obtained from the kernels of the fruit of the tree of Cayor (*Neocarya macrophylla* Sabine).

The seeds of *A. alboviolaceum* (Ridl.) K. Schum oil extract can be used directly in food or feed as the source of carbohydrate.

Rate of proteins

The average protein content has been determined from 6 tests. So we got a 5.19%, low value compared to the almonds of coconut palm (*C. nucifera*) which have a protein content of 13% (www.information nutritionnelle.fr) and the almond tree, which has a protein levels ranging from 18.1 to 21.2%. This value of 5.19% is slightly less than 6.9% (Kabiru et al., 2015) value obtained from *Borassus aethiopum*. The protein content of the seeds of *A. alboviolaceum* (Ridl.) K. Schum studied is 5.19%. This value is very low compared with the seeds of *Parkia biglobosa* (Jacq.) (24.33-33.70%) (Koura et al., 2014); very low compared to the values obtained by some authors working on some varieties of peanuts: 19.81% (Ayoola and Adeyeye, 2010) 27.54-32.85% 23.62-28.88% (Eshun et al., 2013; Mora-Escobedo et al., 2015) 32.64% (Ossoko, 2017). It can thus be said that *A. alboviolaceum* (Ridl.) K. Schum is no protein. The seeds of *A. alboviolaceum* (Ridl.) K. Schum are not a good source of protein.

The seeds of *A. alboviolaceum* (Ridl.) K. Schum are less rich in protein than seeds of *Voandzou* (*Vigna subterranea* (l.) grown in Côte d'Ivoire with a rate ranging

from 14.61 to 20.74% (Diallo et al., 2015)).

Rate of ash and minerals

Different tests for the analysis of the rate of ash gave an average value of 1.18%, lower value than almonds of *C. nucifera*; it hovers around 2.5% (www.fao.org) and to the almond trees which is 2.65%. This indicates that the seeds of *A. alboviolaceum* (Ridl.) Lenaerts Schum) contain less minerals than *C. nucifera* and almond trees, but remain a significant source of minerals. This value is roughly equal to 1.17% (Eshun et al., 2013), value obtained from *Borassus aethiopum*. It is however lower than 4.08%, value obtained from the melon seeds (*Cucumis melo* l. *Inodorus*) (Bouazzaoui et al., 2016) and those ranging from 1.38 to 1.48% from the seeds of peanut (Ayoola and Adeyeye, 2010; Ayoola et al., 2012). It is also lower than those obtained from peanuts by some authors (values ranging from 2.45 to 2.96%) (16). Peanut '*Manga"* has a rate of 5.68% ash (Ossoko, 2017), very high value than that of the seeds studied here. *A. alboviolaceum* (Ridl.) Lenaerts Schum contains less minerals ions (ash 1.18% rate) as *B. aethiopum* (1.60%). Phosphorus, iron, calcium and magnesium were obtained from the ash and the result obtained is as follows: phosphorus: 0.14%; iron: 0.00%; Calcium: 0.72% and Magnesium: 0.29%. This result shows that there are still a lot of minerals to determine in these ashes. These identified minerals are essential for the proper functioning of the body.

Rate of total carbohydrates and dietary fibre

The value of 52.37% obtained is low compared to 81% value obtained from *B. aethiopum* (Kabiru et al., 2015). The levels of carbohydrates of some varieties of peanut: 17.41% (Ayoola and Adeyeye, 2010; Ayoola et al., 2012). 11, 54-19.65% (Eshun et al., 2013) and 17.56% (Ossoko, 2017), are lower than that of *A. alboviolaceum* (Ridl.) K. Schum, which is 52.37%. The seeds of *A. alboviolaceum* (Ridl.) K. Schum are a good source of carbohydrates.

The rate of fiber was 3.86%; very low value compared to 11.2% value obtained from the Palm (*Borassus aethiopum*) (Kabiru et al., 2015). The seeds of *A. alboviolaceum* (Ridl.) Lenaerts Schum) are not a good source of fiber from the *B. aethiopum*.

Energy value (EV)

The energy value obtained is 325.46 Kcal / 100 g. This value is less than those obtained from the seeds of seven cultivars of voandzou (*Vigna subterranea* (l) Verdc. Fabaceae) grown in Côte d'Ivoire, values ranging from 370.02 to 388.8 Kcal / 100 g (Diallo et al., 2015). This value of 325.46 Kcal / 100 g is slightly higher than the *B. aethiopum* (308.87 Kcal / 100 g); seeds of *A. alboviolaceum* (Ridl.) Lenaerts Schum are a good source of energy.

Conclusion

As part of the development of seeds, *A. alboviolaceum* (Ridl.) Lenaerts Schum) which has been the subject of our study is one of many varieties of fruit that exist in our country; there has never been a comprehensive scientific study on it. This study enabled us to achieve this goal by determining the physico-chemical composition of the seeds of the maniguette (*A. alboviolaceum* (Ridl) K Schum) whose results are as follows: water (30.68%), lipids (10.58%), proteins (5.19%); carbohydrates (52.37%); ash (1.18%) and the fibers (3.86%). The values obtained show that these fruits contain significant health nutrients and can be recycled in the industrial production of human foods. The study of food quality oil is to continue the determination of the composition in fatty acids, glycerides, phospholipids, ceramides, and sphingomyelin, the position of fatty acids on triglycerides and phospholipids and the composition of the unsaponifiable. This work should be completed by making a thorough study of the protein fraction of these seeds. Thus, it would enhance oil extract cake of these seeds in food meals and feed manufacturing.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Adegoke BM, Shittu SA, Raimi MM, Oyetade OA, Oyekanmi AM (2014). Effect of Traditional Processing Methods on the Protein and Lipid Content of *Arachis hypogaea* (Groundnut). International Journal of Scientific and Research Publications 4(5):3.

AOAC (2005). Official method of analysis of the Association of official Analytical Chemist, 5th ad. AOAC Press, Arlington, Virginia, USA.

Attibayeba, Ngatsoué L, Massamba D, Makoundou B (2010). Variation des lipides dans les amandes au cours de la croissance et de la maturation des fruits de *Grewia coriaceae* Mast (Tiliaceae). La Rivista Italliana delle sostanse grasse LXXXIII:58-60.

Ayoola PB, Adeyeye A (2010). Effect of Heating on Chemical Composition and Physico-Chemical Properties of *Arachis hypogea* (Groundnut) Seed Flour and Oil. Pakistan Journal of Nutrition 9(8):751-754

Ayoola PB, Adeyeye A, Onawumi OO (2012). Chemical evaluation of food value of groundnut (*Arachis hypogaea*) seeds. American Journal of Food and Nutrition 2(3):55-57.

Balla A, Baragé M (2008). Analyses physico-chimiques de la pulpe et caractérisation de la fraction lipidique des amandes du fruit du pommier de Cayor (*Neocarya macrophylla* Sabine). Bulletin de la Recherche Agronomique du Benin 61:6.

Binaki AF, Kama Niamayoua R, EnzongaYoca J, Loumouamou BW, MvoulaTsieri M, Silou T (2013). Caractérisation Physico chimique de la matière grasse de *Anisophyllea quangensis* Ex Henriq du Bassin du Congo. Journal of Animal and Plant Sciences 20(1):3079-3092.

Bouazzaoui N, Drici W, Bouazzaoui W, Lemerini W, Arrar Z,

Bendiabdellah D, Mulengi KJ (2016). Fatty acids and mineral composition of melon (*Cucumis melo* L. *Inodorus*) seeds from West Algeria. Mediterranean Journal of Chemistry 5(1):340-346.
Brintha I, Mahendran T, Seran TH (2014). Nutritional Composition and Storage Stability of Groundnut (*Arachis hypogaea* L.) Seeds Cultivated with Organic Fertilizers. Tropical Agricultural Research and Extension 17(2).
Diallo KS, Koné KY, Soro D, Assidjo NE, Yao KB, Gnakri D (2015). Caractérisation Biochimique et Fonctionnelle des Graines de Sept Cultivars de Voandzou (*Vigna Subterranea* (l.) Verdc. Fabaceae) Cultivés en Côte d'Ivoire. European Scientific Journal 11(27):2-17.
Eshun G, Adu AE, Barimah J (2013). Nutrients content and lipid characterization of seed pastes of four selected peanut (*Arachis hypogaea*) varieties from Ghana. Global Journal of Food Science and Technology 1(1):108-114.
Jean WH, Yong, Liwa Ge, Yan Fei Ng, SweNgin Tan (2009). The Chemical Composition and Biological of Coconut (*Cocos nucifera*) Water Molecules 14:5144-5164.
Kabiru Jega Umara, Bello Mani Abdullahib, Badaru Muhammadc, Sirajo Muhammadd, Lawal Gusau Hassane, Nasiru Alhaji Sanif, (2015). Nutritional and Antinutritional Profile of *Borassus aethiopum* Mart (African Palmyra Palm) Shoots. International Journal of Sciences Basic and Applied Research, pp. 1-11.
Kapseu C (2009). Production, Analyse et Application des huiles végétales Africaines. OCL 16:215-229.
Koura K, Ouidoh GIP, Azokpota P, Ganglo CJ, Hounhouigan JD (2014). Caractérisation physique et composition chimique des graines de *Parkia biglobosa* (Jacq.) R. Br. en usage au Nord-Benin. Journal of Applied Biosciences 75:6239-6249.
Loumouamou B. (2012). Contribution à la valorisation des oléagineux du genre *Irvingia* du Bassin du Congo. Composition chimique et potentialités technologiques des amandes, Thèse de Doctorat de l'Université Marien Ngouabi, Brazzaville, 128p.
Mora-Escobedo R, Hermandez-Luna P, Joaquin-Torres IC, Ortiz-Moreno A, Robles-Ramirez M. Del C. (2015). Physicochemical properties and fatty acid profile of eight peanut varieties grown in Mexico. CyTA-Journal of Food 13(2):300-304.
Mustapha S, Mohammed UM, Adeosun NO, Mathew TJ, Muhammed SS, Ibn-Aliyu A (2015). American Journal of Food Science and Technology 3(5):126-131.
Ngakegni LCA (2012). Etude de synergie des effets chimiques et biologiques des lipides de réserves et des huiles essentielles des fruits et graines saisonniers de la sous-région Afrique Centrale. Thèse, Université de Toulouse, 170p.
Olayinka BU, Yusuf BT, Etejere EO (2015). Growth, Yield and Proximate Composition of Groundnut (*Arachis hypogaea* L.) as Influenced by Land Preparation Methods. Notulae Scientia Biologicae 7(2):227-231.
Ossoko JPL (2017). Valeur Nutritionnelle des Arachides (*Arachis hypogaea* L.) de «*MANGA*» : Etude de leurs Propriétés Lipidiques et Allergéniques. Thèse de Doctorat Unique. 142p.
Silou T (2014). Corps gras non conventionnels du Bassin du Congo: Caractérisation, biodiversité et qualité. OCL 21(2)D209:15.
Silou T, Biyoko S, Heron S, Tchapla A, Maloumbi MG (2004). Caractéristiques physico-chimiques et potentialités technologiques des amandes de *Irvingia gabonensis*. La Rivista Italiana Dell Grasse (LXXXI):49-56.
Tapia IM, Sanchez-Morgado RJ, Garcia-Parra J, Ramirez R, Hernandez T, Gonzalez-Gomez D (2013).Comparative study of the Nutritional and Bioctive compounds content of four Walnut (*Juglans regia* L.) cultivars. Journal of Compsition and Analysis 31:232-237.
Wolff JP (1968). Manuel d'analyse des corps gras; Azoulayéd., Paris (France), 519 p.
Womeni HM, Tiencheu B, Tenyang N, Tchouanguep Mbiapo F, Kapseu C, Linder M, Fanni J (2011). Extraction of palm kernel oil in Cameroon: effets or kernels driying on oil quality. Journal of Food Technology 8:1-7.

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