ORIGINAL PAPER

Nig. J. Pharm. Res. 2016, 12 (2) pp 87-94
ISSN 0189-8434 **Available online at http://www.nigjpharmres.com**

GC–MS Profile, Anti-Seizure and Anti-Pyretic Activities of Palm Kernel Nut Oil and its Isolate, N-Octanoic Acid from Specially Breed Palm Kernel *Elaeis Guineensis*

*C. S. Alaribe[1ACDEF], C. Anyakora[1EF], E. Emoghene[1BDF], D. Ota[2BCF] and M. De Waard[3F]

[1] Dept of Pharmaceutical Chemistry Faculty of Pharmacy, University of Lagos, Nigeria
[2] Dept of Physiology, College of Medicine, LUTH, University of Lagos, Nigeria
[3] Institute de Neuro Science de Grenoble, France

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

PURPOSE: Palm kernel nut oil (PKNO) from palm fruit of a rare breed of *Elaeis guineensis* (*virescens*), and one of the species belonging to the *Elaeis* genre of *arecaceae* family was subjected to Gas chromatography - Mass spectroscopic studies as well as anti-seizure and anti-pyretic studies. It is used in South-Eastern part of Nigeria as antipyretic and anti-seizure agents especially in children. The aim of this study was to evaluate the chemical contents of PKNO using GC-MS and to investigate the antiseizure and antipyretic properties of PKNO using animal model.

METHOD: 1500 g Palm Kernels (PK) of the rare breed collected from Abia State were cracked open and the nuts (1000 g) poured into steel vessel and heated until oil was produced after charring the nuts. The oil (PKNO) was analyzed using GC-MS to determine its chemical composition. Yeast induced hyperthermia method was used for anti-pyretic studies while strychnine sulphate induced method was used for anti-seizure studies. PKNO and Octanoic acid (0.5 mg/kg ,1 mg/kg, 2 mg/kg) were administered intraperitoneally (IP).Paracetamol (0.15mg/kg) was used as reference standard for anti-pyretic assay while epilim and tegretol (0.14mg/kg, resp.) were used as reference standards for anti-seizure assay.

RESULTS: The major component identified in the extract include: Dodecanoic acid,2,3-dihydroxypropyl ester(19.36 %),n-Hexadecanoic acid(15.49 %),Dodecanoic acid(12.51 %),Myristic acid (6.47 %), Dodecanedioic acid (3.93 %),n-Acetylpyrrolidone (3.67 %) , Thiazole (0.98 %) and Octanoic acid, OA (3.19 %).The anti-seizure results showed that doses of PKNO and OA used had Significant ($p<0.02$) anti-seizure activities by delaying the on-set of seizure from time of induction to time of first seizure observed. In yeast induced hyperthermia model, PKNO exhibited good level of anti-pyretic activities by direct reduction of pyrexia with the highest dose, 2.0 mg/kg. The PKNO and OA are proven to be safe till 3000 µl/kg as indicated by LD_{50} results.

CONCLUSION: PKNO and its components mainly fatty acids, thiazole, and n- acetylpyrrolidone are potent and promising agents with anti-pyretic and antiseizure activities.

Keywords: Anti-Seizure, anti-pyretic, Octanoic acid, *elaeis virescens*, GC-MS, Palm kernel nut oil (PKNO)

INTRODUCTION

Infant and childhood mortality rates have remained very high in developing countries with one in every six African child dying before the age of five [UNICEF. New York 2009; Bryce J and Requejo JH., 2010]. Febrile seizure is one of the most common seizure disorders in childhood and contributes to the causes of infant/childhood mortality especially due to negligence and poor health care. Statistics of occurrence varies in Nigeria with values ranging from 10 - 18 %. [Eseigbe EE, *et al*, 2012; Jones T and Jacobsen SJ. 2007]. Seizures generally are neurological disorder in which clusters of nerve cells, or neurons in the brain sometimes signal abnormally and the disturbances resulting to strange sensations, emotions, and behaviors. These disorders can also cause convulsions, abnormal movements and loss of consciousness. Seizures often accompanied with febrile conditions is the most common childhood neurological disorders with a worldwide incidence of 1- 14 % [Hauser WA., 1994] and the second most common neurological disorder causing early death. The correlation between fever and seizure and their pathophysiology is not very clear but a family history may suggest genetic susceptibility.

Seizures have been managed successfully with anti-convulsion drugs (ACDs) but not without being linked to various side effects including neurotoxicity thereby limiting most of their uses. On the contrary many medicines of plant origin are being used to treat various ailments with little or no adverse effects. It is therefore essential that continuous effort is made to develop more effective, cheaper and safer medicinal agents. It is for this reason that we investigated the oil extract from special breed of *elaeis guineenis (virescens)* as a potential anti-convulsion agent as claimed by indigenous users in South – East, Nigeria.

Plants represent a large natural source of useful compounds that might serve as lead for the development of novel drugs. Palm kernel nut (PKN), known as *Nkwu - Obia* in Abia State, South Eastern part of Nigeria, is a rich medicinal plant whose oil extract from the nuts is used traditionally and extensively for treatment of high fever and convulsion especially in children.

Even with the extensive forkloric use of PKNO for medicinal uses, phytochemical and biological evaluations studies are yet to be carried out on this specie of palm kernel nut to the best of our knowledge. But a few studies have shown that Palm fruit oil contain high level of fatty acids and their esters (Atasie VN and Akinhanmi TF, 2009). In this study, some chemical constituents of PKNO using gas chromatography – mass spectroscopy, their anti-seizure and anti-pyretic properties were investigated.

MATERIALS AND METHOD

Collection of plant material

The Palm nut fruits of *elaeis guineensis* (virescens) were obtained from Ikwuano in Abia State. The family and generic name was identified by Mr. Reinout Impens, the Research Development Manager in PRESCO, an agro industrial establishment with specialty in oil palm plantations located in Benin City, Edo State. Nigeria.

Extraction and preparation

Sufficient quantity of palm kernel nuts (PKN) of *elaeis guineensis* (virescens) were washed with clean water, sun dried and stored in a cool area of a room. 1000 g of PKN poured in a clean steel vessel were set on combustion at temperature above 100^{o}C. After about 20 mins of continuous stirring, the kernels got charred producing dark brownish oil with mild soothing aroma. The process was continued until all the nuts have been charred. The palm kernel nut oil (PKNO) was collected, filtered and properly stored in a clean and dry container prior to use.

Gas chromatography – mass spectrometry analysis (GC - MS)

GC-MS analysis was carried out at the Central Research Laboratory, University of Lagos, Nigeria.

Approximately 10 µl of PKNO was dissolved and diluted in 0.5 ml hexane and dried in anhydrous sodium prior to GC - MS analysis. The analyses were performed on Agilent capillary 7890A gas chromatograph directly coupled to the mass spectrometry detector 5975C. HP – 5 MS, non-polar fused silica capillary column (30 m x 0.25 mm, 0.25 µm film thickness) was used. The oven temperature was set at 70^{o} C for 4 min and ramped at 8^{o} C/min to final temp 240^{o} C and held for 20 min. The flow rate of the carrier gas, Helium, was 1mL/min. The injection volume was 1 µL of diluted oil in hexane. The mass spectrometer detector was used in electron ionization mode and all spectra were acquired using a mass range of 50 to 550 m/z and Automatic Gain Control (AGC). The identification of compounds was based on the retention time match and mass spectra

match against standards and NIST mass spectra library.

Selection of animals and handling:
Healthy Sprague – dawley rats of both sexes weighing between 75 – 110 g obtained from the animal house of the University of Lagos were used for toxicity, anti-seizure and anti-pyretic studies. The rats were housed in a well-ventilated animal house under a controlled light, temperature and humidity conditions. They also had a free access to standard feed and water. The rats were fasted for 24 hours and placed in various groups of five rats each to facilitate experimental work and for accurate evaluation. Handling of animals was done in accordance with international acceptable guidelines and approval from College of Medicine, University of Lagos Ethical Committee.

Acute toxicity Test
The acute toxicity test for PKNO and Octanoic acid (OA) were determined in rats. The medial lethal dose of the extract that can kill 50 % of the animals in a population (LD_{50}) was evaluated. The animals were fasted 3 hrs prior to the experiment, after which single doses of PKNO were administered **intraperitoneally** to the four different groups (1000, 2000 3000, 5000 µl /kg) with five rats in each group. The rats were observed for 48 hrs. LD_{50} test was also carried out with Octanoic acid using only 3000 µl /kg.

Anti –pyretic activities
Yeast induced hyperthermia was used to investigate the anti-pyretic activities in this study. The normal body temperature of each rat was measured rectally using thermometer at predetermined intervals of **30 mins** and recorded. The test was performed in rats by injecting 10 ml/kg, s.c., of 15 % aqueous solution of yeast in 0.9 % saline solution to induce pyrexia. Rectal temperature of each animal was taken before and 19 hrs after the yeast injection using digital clinical thermometer. Only animals displaying a mean basal rectal temperature above 36.8 ºC were selected for the study. The animals were picked up gently and held manually during the temperature measurements. This procedure was performed at least twice on the day before the experiment to minimize changes secondary to handling. The experiment was carried out in a temperature-controlled room (32ºC) by ensuring proper ventilation. All animal experiments strictly complied with the approval of College of Medicine Ethical Committee, University of Lagos.
The rats were divided into 7 groups consisting of five animals each and housed in polyacrylic cages. Group - I: Control (distilled water); Group - II: reference standard (Paracetamol; 0.15mg/kg); Group - III: PKNO (2mg/kg); Group - IV: PKNO (1mg/kg); Group - V: PKNO (0.5mg/kg); Group -VI : OA (2mg/kg) and Group – VII: OA (0.5mg/kg).

Strychnine induced convulsion test
The anti-seizure activities of PKNO and Octanoic Acid (OA) were carried out using a modified method of Amabeoku and Chikuni [Amabeoku, GJ, and Chikuni, O.; 1993]. Male albino rats weighing between 75-100 g were used for this study. Strychnine sulphate, $(C_{21}H_{22}O_2N_2)_2.H_2SO_4.5H_2O$ (1 mg/kg) was the convulsing agent used to induce seizure in rats before (pre) and after (post) treatment with test drugs. Epilim and tegretol (0.14 mg / kg respectively) were used as reference standards. The rats were randomly allotted to different groups of five rats in each group; control with distilled water (group I) and test groups with graded doses of PKNO ({Group II, III, IV}, groups VIII, IX and X), OA (Group V), epilim (group VI) and tegretol (group VII). The animals were subjected to 3 hours fasting before the experiment. The animals in groups II, III and IV received; 0.25 mg/kg, 0.5 mg/kg and 1 mg/kg and kept for 45 mins before the administration of strychnine sulphate (Pre-treatment). Another set of animals in groups VIII, IX and X were also treated with **PKNO** (0.25 mg/kg, 0.5 mg/kg and 1 mg/kg; respectively) after strychinine sulphate was induced (Post treatment). Onsets, duration of seizure, as well as percentage mortality were recorded as the animals were observed closely.

Statistical analysis:
The data were expressed as mean ± S.D. Where applicable the difference in response to test drugs was determined by Students's t-test. $P < 0.05$ was considered significant.

Results

GC-MS Data results
GC - MS analysis of PKNO revealed high level of fatty acids and their esters as expected. Figure 1 shows the total ion chromatogram of PKNO. Table 1 shows the Retention time and relative content of PKNO expressed as percentage from the total area. From the results, it can be seen that 36 compounds with over 0.15 percent area were separated but only 20 adding up to 98.49 % of the total area were identified. The major components identified are dodecanoic acid (12.50 %), n-Hexadecanoic acid (15.50 %), dodecanoic acid, 2, 3-dihydroxypropyl ester (19.36 %), N-acetyl pyrrolidone (3.67 %) and octanoic acid. (3.19 %). This result corroborates an earlier study by Atasie and Akinhanmi [6] on

chemical components of palm fruit oil. One of the components, Octanoic acid (OA), a medium chain triglyceride has some important pharmacological activities.

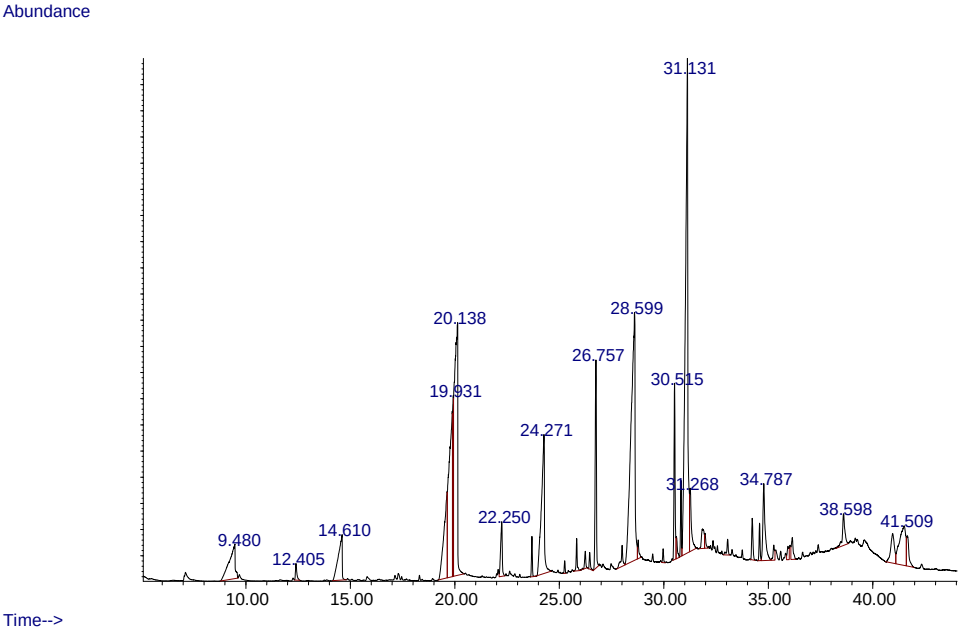

Figure 1: chromatogram of oil extract from *elaeis guineensis (virescens)*

Table 1: Chemical composition of PKNO from *elaesis giuneesnsis* (*virescens*).

No	*Compound*	*RT (min)*	*Relative content (%)*
1.	Octanoic acid	9.48	3.19
2.	n-Decanoic acid	14.61	2.69
3.	Dodecanoic acid	19.88	12.50
4.	Tetradecanoyl chloride	23.70	0.54
5.	Tetradecanoic acid	24.27	6.48
6.	Dodecanedioic acid	26.76	3.93
7.	n-Hexadecanoic acid	28.60	15.50
8.	Naphthalene,1,2,3,4-tetrahydro-1-methoxy	30.52	2.93
9.	15-Hydroxypentadecanoic acid	30.82	1.03
10.	Dodecanoic acid,2,3-dihydroxypropyl ester	31.13	19.36
11.	Hexadecanoic acid, 2,3-dihydroxypropyl ester	31.27	1.74
12.	Cis – Vaccenic acid	31.86	0.34
13.	L-Homoserine,N,O-dimethyl-,methyl ester	34.23	0.80
14.	Oxalic acid,allyl tridecyl ester	34.59	0.56
15.	1,3-O-Benzyllidene glyceryl -2-myristate	34.79	2.60
16.	Pyrrolidine, 1-(7-oxo-2,4,6-trimethylheptanoyl)	36.15	0.59
17.	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester	38.60	1.25
18.	Eicosane, 10-methyl	40.96	1.64
19.	N-Acetylpyrrolidone	41.51	3.67
20.	Thiazole , 5-methyl	41.66	0.98
	Total Identified Constituents		**98.49**

RT : Retention time obtained from the chromatogram (Fig 1)
M.m : Molecular mass

Toxicity Results

The acute toxicity study of PKNO and OA were carried out in rats and lethal dose (LD_{50}) determined. The results in table 2 showed that PKNO and OA are safe up till 3000 µl/kg.

Table 2: Toxicity results of PKNO in animal (rat) model

Treatment	(Dose) µl/kg	Deaths
PKNO	5000	5/5
PKNO	3000	0
PKNO	2000	0
PKNO	1000	0
OA	3000	0

Results of test materials on yeast induced hyperthermia in rats

The result of the effects of test materials: PKNO, OA and Paracetamol (PCM) on yeast induced hyperthermia carried out in rats are shown in Figures 2 and 3.

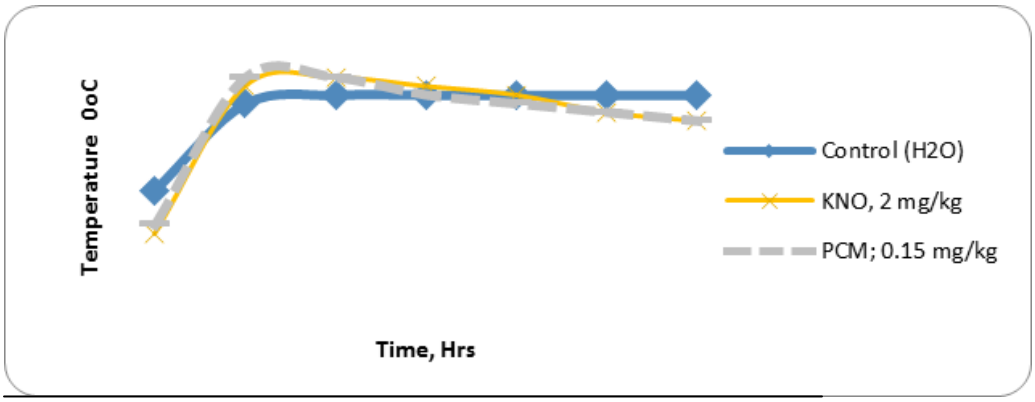

Figure 2: Effects of Control (H_2O only), PKNO (2 mg/kg) and PCM on yeast induced hyperthermia in rats

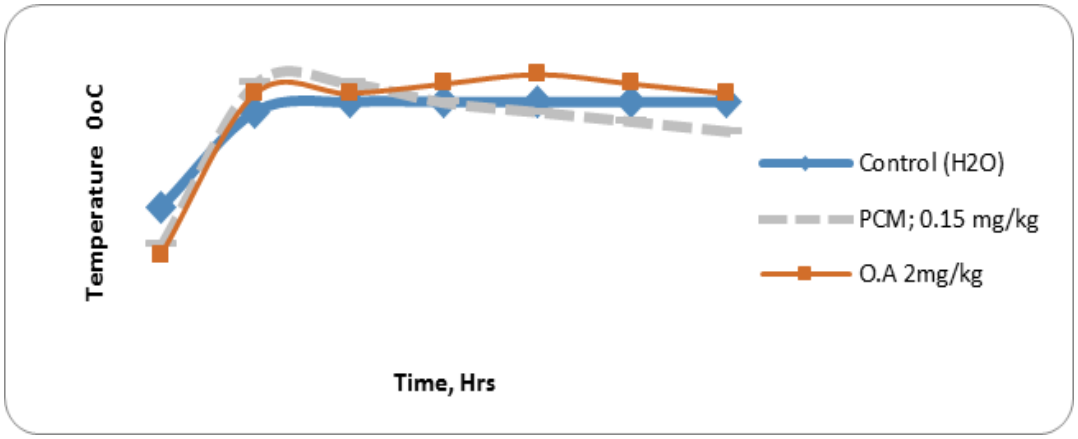

Figure 3: Effects of Control (H_2O only), Octanoic acid(O.A) (2 mg/kg) and PCM on yeast induced hyperthermia in rats

Administration of yeast through subcutaneous route in all groups of animals subjected for this assay caused elevation of rectal temperature by range of 38.1 to 39.9 ^{o}C (Δ 1-1.99^{o}C) as observed **between To - T**19 hrs in figures 2 and 3. PKNO showed a dose dependent direct reduction of pyrexia, with the highest dose (2.0mg/kg) decreasing the rectal temperature from 39.9 to 39.4 (Δ 0.4 ^{o}C) as shown in Figure 2 and minimal difference observed for lower doses (1.0 mg / kg and 0.5 mg/kg). Octanoic acid, an isolate of PKNO did not show any reduction in temperature elevated by yeast in the rats, Figure 3.

Effect of test materials (PKNO, OA, Epilim and Tegretol) on Strychnine sulphate induced seizure

Table 3: Effects of test materials (PKNO, OA, Epilim and Tegretol) on Strychnine sulphate induced seizure in rats – pretreatment method

Treatment	(Dose) mg/kg	Time of Ss injection (am)	Onset of seizure (am)	Duration of seizure (mins)	Deaths	Protection from mortality (%)
control (H_20)	-	08.20	08.24	4	5/5	0
PKNO	0.25	08.22	08.30	8	5/5	0
PKNO	0.50	09.47	10.54	7	5/5	0
PKNO	1.00	10.00	10.10	10	1/5	1
OA	1.00	10.20	10.25	5	5/5	0
Epilim	0.14	10.40	10.50	10	5/5	0
Tegretol	0.14	10.45	10.54	9	5/5	0

Ss: *Strychnine sulphate* *vehicle control* ***(H_2O only)***

The test materials used here were PKNO, OA, epilim and tegretol. Strychnine sulphate $(C_{21}H_{22}O_2N_2)_2.H_2SO_4.5H_2O$ was used to induce seizures in all groups of animals subjected in this study. The convulsing action of strychnine has been suggested to be due to interference with post synaptic inhibition mediated by glycine, an important inhibitory transmitter to motor neurons and inters neurons in the spinal cord. The use of strychinine sulphate as a seizure agent is associated with mild behavioural signs in the rats with continued stimulation of electrical activities which presumably spread resulting to generalized seizures. Although the pathogenesis of kindled seizures is not fully understood in animal model, it serves as a useful tool for investigating the efficacy of experimental anti-seizure agents.

All the doses of test materials, used did not interfere with the occurrence of strychinine sulphate elicited seizures for pre-treatment method. However, we observed that the all doses of PKNO in pre-treatment procedure significantly ($p<0.02$) delayed the onset of seizures when compared with the vehicle control having distilled water only as shown in table 3. There were no results for post treatment procedures as the experiment was not conclusive.

DISCUSSION

The oil extracted from Palm Kernel Nut (PKN) of rare breed specie, *elaeis guineensis*, (*virescens*) was analyzed using GC-MS and constituents identified by comparing the obtained mass spectra with those of authentic standards and spectral library from NIST. Results based on their relative contents (%) show that PKNO contains appreciable amount of fatty acid and their esters. Major components include; octanoic acid, dodecanoic acid, tetradecanoic acid, dodecanedioic acid, n-hexadecanoic acid, dodecanoic acid, 2,3-dihydroxypropyl ester and n-acetylpyrrolidone according to NIST library (Chem Station, USA. 2014). Fatty acids are important sources of fuel because when metabolized, they yield large quantities of ATP most especially to the heart, skeletal muscle and brain cells. One of the fatty acid isolate of PKNO, Octanoic acid (OA), has been indicated in the treatment of allergy, hypersensitivity and epilepsy. It is also been suggested as an alternative energy source for brain cells that have lost their ability to use glucose (sugar) as a result of some disease conditions such as Alzheimer's disease [Elbert, D *et al* ; 2003]. It is interesting to observe a level of positive results for anti pyretic and anti seizure activities for some doses of PKNO and it's recommended that further investigations be carried on especially with higher doses.

Other components present in PKNO are thiazoles and pyrrolidine, 1-(7-oxo-2, 4, 6-trimethylheptanoyl) though not relatively in abundance as those previously mentioned but have been reported to possess significant medicinal activities. This is the first time they are being reported present in PKNO using GC-MS. Agents affecting the strychnine induced pathway can inhibit or slow down seizures in pre – treated model of the assay. The pre-treatment assay showed significant ($p<0.02$) delay to the onset of seizures for all doses of test materials comparable to the vehicle control with distilled water only, though the mechanisms of activities are not clear. However, the presence of Thiazoles in PKNO may be contributory in its anti-seizure activities. They are compounds containing azomethine group (–C=N–) and their derivatives constitute an important family of heterocyclic compounds [Sooryanarayana Rao B *et al* , 2000]. Compounds bearing this nucleus are found in the drug development for the treatment of allergies, inflammation, HIV infections and hypnotics. They also exhibit remarkable biological activities such as antimalarial, anticancer and anticonvulsant [Siddiqui, *et al* ; 2009; Zarghi, , *et al* , 2005].

Another interesting component of PKNO identified by GC-MS is Pyrrolidine moiety. Previous studies have shown levetiracetam of Pyrrolidine moiety to be effective in the treatment of epilepsy though the exact mechanism of action is not yet known but it appears to slow down nerve transmission by binding to the synaptic vesicle protein SV2A which is thought to be involved in the regulation of vesicle exocytosis. [Laxmikant and Robert 2014]. They are used as adjunct therapy to treat partial and tonic-clonic seizures. The presence of Pyrrolidine in PKNO is also suggestive of its potential as anti-seizure drug.

A considerable anti-pyretic effect of PKNO especially with higher dose (2 mg/kg) is commendable. Fever is one of the frequent indications of most illnesses and most seizures are usually preceded by fever. When these seizures are caused by a fever, they are called febrile seizures (FS). The height of the fever and the rapidity of the elevation of temperature are both involved in triggering a seizure and as a result, typical treatment of FS is to foremost reduce temperature by antipyretics and passive cooling. PKNO and OA, demonstrated moderate antipyretic activities as apparent in the inhibition of temperature elevation and a direct reduction of pyrexia in the yeast induced model. The mode of action is yet to be studied but its antipyretic action is suggested to be by inhibition of the enzyme cycloxygenase (COX), reduction in the levels of prostaglandin E_2 (PGE_2) within the hypothamus, their ability in the reduction of pro inflammatory mediators or enhancement of the anti-inflammatory signals at sites of injury or even to boost antipyretic messages within the brain. (Aronoff D.M and Neilson E.G, 2001)

The acute toxicity studies showed that PKNO and OA are relatively safe until doses > 3000 µl / kg as shown in the LD_{50} results. It was also observed that at doses above 3000 µl / kg, the animal started passing watery faeces. This finding is also an interesting finding of PKNO which can validate one of the folkloric claims of PKNO as purgatives and need to be investigated further. The peculiar extraction method of PKNO is also suggestive to be contributory to its wide medicinal properties.

CONCLUSION

Palm kernel nut oil (PKNO) extracted by charring the nuts was subjected to GC-MS analysis which revealed the presence of twenty identified compounds. PKNO and one of its isolates, OA are suggested to be potential anti-pyretic and anti-seizures agents. Their mechanisms of action are yet to be investigated.

Acknowledgement

The authors are grateful to Mejida Sumaila and Mrs Olafumihan of the Central Research Lab, University of Lagos, Akoka for their assistance in GC-MS techniques. The authors are also grateful to Lady Ahaoma Anyim for providing the palm kernel nut extract.

REFERENCES

Amabeoku, G.J and Chikuni, O. (1993). Cimetidine – induced seizures in mice. Biochem. Pharmacol. 46 (12): 2171 -2175.

Aronoff, D.M and Neilson, E.G. (2001). Antipyretic: mechanisms of action and clinical use in fever suppression PubMed, National Institute of Health. 111 (4): 304 -15.

Atasie, V.N and Akinhanmi T.F. (2009). Extraction, Compositional Studies and Physico-Chemical Characteristics of Palm Kernel Oil. Pakistan Journal of Nutrition. 8: 800- 803.

Bryce J and Requejo J.H. (2010). Tracking Progress in Maternal, Newborn and Child Survival: The 2010 Report. New York; UNICEF.

Elbert, D., Haller, R.G., Walton, M.E. (2003). Energy contribution of octanoate to intact rat brain metabolism measured by ^{13}C nuclear magnetic resonance spectroscopy. The Journal of neuroscience. 23 (13): 5928–5935.

Eseigbe, E.E., Adama S.J., Eseigbe P. (2012). Febrile seizures in Kaduna, north Western Nigeria. Nigerian Medical Journal, NMJ. 53 : 140 – 144.

Hauser, W.A. (1994). The prevalence and incidence of convulsive disorders in children. Epilepsia. 35 : 3; 1 – 6.

Jones, T and Jacobsen, S.J. (2007). Childhood Febrile Seizures: Overview and Implications. Int J Med Sci. 4: 110 – 114.

Laxmikant, S.D and Robert, J.D. (2014). Mechanism of Levetiracetam in the Control of Status Epilepticus and Epilepsy. Front Neurol. 4: 5- 11.

Siddiqui, N., Faiz Arshad, M., Ahsan W and Alam, M.S. (2009). Thiazoles: a valuable insight into the recent advances and biological activities. International Journal Pharmaceutical Sciences and Drug Research. 1 : 3; 136 –143.

Sooryanarayana Rao, B., Shridhara K and Akberali, P.M. (2000). Studies on arylfuran derivatives: part XI. Synthesis, characterisation and biological studies on some Mannich bases carrying 2, 4-dichlorophenylfurfural moiety, Farmaco. 55: 5; 338–344.

UNICEF. State of the World's Children 2010. New York 2009; UNICEF.

Zarghi, A., Tabatabai, S.A, Faizi, M. (2005). Synthesis and anticonvulsant activity of new 2-substituted-5-(2-benzyloxyphenyl)-1, 3, 4-oxadiazoles. Bioorganic and Medicinal Chemistry Letters. 15: 7; 1863–1865.

*Address for correspondence:

Chinwendum. S. Alaribe

Dept of Pharmaceutical Chemistry Faculty of Pharmacy, University of Lagos, Nigeria

Telephone: **+234-8037263962/+234-8174633001**

E-mails: **salaribe@unilag.edu.ng / amachichi1@yahoo.com**

Conflict of Interest: None declared

Received: 10 August, 2016

Accepted: November 16, 2016