Effect of *Cocos nucifera* and *Elaeis guineensis* oils on the hypoglycaemic activity of metformin on alloxan-induced diabetic rats

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ABSTRACT

Background*:* Plant extracts are known to be rich in phytochemicals that possess pharmacological properties that are usually deployed in the management of some physiological imbalances in humans including diabetes mellitus. These bioactive compounds sometimes combine with formulated drugs in concomitant administration to give a number of unimaginable outcomes in some instances. The aim of this research work was to evaluate the hypoglycaemic potentials of *Cocos nucifera* and *Elaeis guineensis* oils and their effects when combined concurrently with metformin on alloxan-induced diabetes mellitus in rat.

Methods*:* Virgin coconut oil (VCO) and palm kernel oil (PKO) were extracted using the hot extraction process. Diabetes was induced in the rats with alloxan and the blood glucose level was determined using a glucometer.

Results*:* The result of the analysis revealed that VCO and PKO yielded 15.75 % and 13.50 % w/w oil, saponification values of 267.712 ± 0.07 mgKOH/g and 361.377 ± 1.69 mgKOH/g and acid value of 0.804 ± 0.09 mgKOH/g and 11.088 ± 0.09 mgKOH/g, respectively. The hypoglycaemic result revealed that the daily dose of PKO was able to cause a reduction in mean blood glucose level relative to negative control, bringing blood glucose level down from 509 mg/dL to 196 mg/dL within 7 days of administration, while,VCO drastically brought down glucose level but could not reduce it to the normal level within 14 days.

Conclusion*:* The concomitant administration of VCO and PKO with metformin showed synergistic and antagonistic property, respectively.

***Key words*— Alloxan-induced diabetes, *Cocos nucifera* oil, *Elaeis guineensis* oil, hypoglycaemic activity, metformin.**

1. INTRODUCTION

Alloxan is one of the common diabetogenic agents often used to assess the antidiabetic potential of pure compounds and plant extract in studies involving diabetes. Its effect usually results in a form of insulin dependent diabetes mellitus commonly referred to as type 2 diabetes [1]. Natural products have been an essential part of healthcare and disease management. Many of these products have been identified, studied and found to be very useful in the treatment and management of several disease conditions. Products of natural resources have a wide range of diversity of multi-dimensional chemical compounds. In recent times, the deployment of natural products as biological function modifiers has also won considerable attention [2], [3], [4]. Oral anti glycaemic agents have been employed extensively in the management and control of type 2 diabetes mellitus with good therapeutic outcomes. However, there are evidences of the use of natural products in the management of this disease and foods rich in lipids have been shown to improve the therapeutic effect of some hypoglycaemic agents. Some of these products are rich in fatty acids especially the medium and long chain fatty acids. VCO and PKO are known to be rich in these classes of fatty acids. These oils are readily available in the local market and can be accessed by low income and high income populace. Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycaemia. The aetiology of diabetes mellitus varies significantly but always includes defects in insulin

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Secretion and response or both in the course of the disease. Mostly, patients with diabetes mellitus have either type 1 diabetes mellitus (which is immune-mediated or idiopathic) or type 2 diabetes mellitus (formerly known as non-insulin dependent diabetes mellitus). Diabetes can also be caused by gestational hormonal environment, genetic defects, other infections and certain drugs [5], [6]. The effect of diabetes can leads to long-term severe vascular complications. Chronic hyperglycaemia affects several organs in the body causing them to dysfunction and failure of the organ may result [7], [8]. The pharmacologic management in patients with type 2 diabetes with intention to achieve glycemic goals, often makes use of agents such as sulfonylureas, meglitinides, metformin, alpha-glucosidase inhibitors, thiazolidinediones, dipeptidyl peptidase IV inhibitors, glucagon-like peptide 1 receptor agonist, and insulin. These agents can be used singly or in combination to achieve targeted glycemic control. A wide range of natural products have also been reported to possess promising glycaemic control, including VCO and PKO. Documented reports opine that both VCO and PKO have exhibited hypoglycaemic activity in test animals due to their antioxidant properties [9]. VCO in particular has been suggested to reduce the severity of islet damage in alloxan induced diabetic rats [10]. There is a great challenge for healthcare providers and research scientist in addressing the increased demands for management of chronic illnesses such as diabetes mellitus. It is therefore imperative that several management strategies be implemented and novel ideas and products introduced to curb or slow down the progress and also improve the patients' quality of life [6], [11]. This has led to the progression in diabetes mellitus management from the use of insulin therapy only, to the use of other means such as oral hypoglycaemic agents, dietary modifications and recently, the use of natural products exclusively or in combination with oral hypoglycaemic agents such as that embarked upon by this study. Hence, this work explored the hypoglycaemic potentials of virgin coconut oil (VCO) and palm kernel oil (PKO) and their effects when combined with hypoglycaemic drug of choice, metformin, in the management of alloxan-induced diabetes in rat.

2. MATERIALS AND METHODS

2.1 Materials

Metformin hydrochloride (Diabond® Metformin Tab 500 mg), manufactured by Bond Chemical Industries Limited, Nigeria, used in this study was purchased from a Pharmacy shop in Uyo metropolis. The reagents used for analysis include potassium hydroxide (May & Baker, England), hydrochloric acid (36% w/v) and ether (BDH Laboratory Reagents., England), ethanol and phenolphthalein indicator (Surechem Products Limited, England), D-(+)-glucose (Otto Chemie PVT Ltd., India) and Alloxan monohydrate (Sigma Aldrich, Germany). The equipment used in the study include weighing balance (Mettler Toledo MS-TS, United Kingdom), oven (T503 – Genlab, England) and water bath (J. P. Selecta 60044, England). The software, Graphpad Prism version 5.0 for Windows (La Jolla California, USA) was used for graph plotting and statistical analysis. Cracked palm kernel and matured coconuts were purchased from Itam main market in Uyo Local Government Area of Akwa Ibom State.

2.2 Methods

2.2.1 Identification of Sample

Palm kernel and coconut were authenticated by Professor Margaret Bassey in the Department of Botany and Ecological Studies, Faculty of Natural and Applied Sciences, University of Uyo. Voucher specimens were deposited in the University of Uyo herbarium. Albino mice and rats were procured from the Deparment of Pharmacology and Toxicology, University of Uyo, Uyo.

2.2.2 Extraction of Oils

2.2.2.1 Virgin Coconut Oil (VCO)

The extraction of VCO was done using the hot extraction method [12]. Briefly, the mature coconuts were dehusked, the shell broken and the endosperm removed. The solid endosperm, 4.0 kg, was grated with a locally fabricated mechanical grater and 3 L of distilled water added to it, stirred to mix properly and filtered with a clean muslin cloth. The residue was rinsed with another 2 L of distilled water and the coconut milk pooled together. The resultant coconut milk was allowed to stand for 24 hours to facilitate the gravitational separation of the emulsion. Demulsification produced three layers; an aqueous phase (water) at the bottom, an emulsion phase (cream) in the middle, and an oil phase on top. The oily and middle creamy layers were decanted and heated at 80 – 100 ^{o}C for about 5 hours. The oil recovered after evaporation of water was decanted into a 500 mL beaker. The VCO obtained was transferred into a clean transparent plastic container, labelled and kept air tight until needed for the analysis.

2.2.2.2 Palm Kernel Oil (PKO)

The hot aqueous extraction method was also used in the extraction of PKO [13]. Exactly 4.0 kg of palm kernel was washed to remove dirt and foreign materials, sieved, dried at 40˚C for 6 hours in an oven, pulverized using manual blender, soaked in 10 L of distilled water and mixed thoroughly. The mixture was heated at between 95˚C – 100 ˚C to excite the oil cells. The hot mixture was transferred into a mesh bag and squeezed. The decoction extract was evaporated in a water bath at 60 ˚C for 36 hours to form two layers of transparent oil and thick brownish residue.

The oil that formed as the upper layer was decanted into a clean air tight plastic container, labelled and kept at room temperature for the analysis.

2.2.3 Determination of Percentage Oil Yield

The percentage oil yield was calculated using the method of Nkafaniya [14]. The total weight of the coconut meat and palm kernel were previously obtained before extraction. The extracted oils were also weighed with an electronic balance and 1 mL of oil was equivalent to 0.947 g. The percentage oil yield was calculated using the formula:

$$Percentage\ yield\ = \frac{weight\ of\ oil\ (g)}{weight\ of\ coconut\ meat\ (g)} \times\ 100$$

2.2.4 Acute Toxicity Study

The Lorke's [15] method was adopted and all (30) albino mice (20 – 25 g)used were sourced from the Department of Pharmacology, Faculty of Pharmacy, University of Uyo. They were fasted overnight prior to the analysis, weighed and marked. The first set of 18 mice were divided into two main groups (A and B) of 9 mice each corresponding to VCO and PKO. In each of the two main groups, the mice were further divided into three sub-groups (1, 2 and 3) of 3 mice each. In the first phase of the study, both the VCO and PKO were administered orally. Exactly 1000 mg/kg, 2,000 mg/kg and 3,000 mg/kg was gavaged to sub-groups 1, 2 and 3, respectively. There was no mortality after 24 hours. In the second phase, another set of 12 mice were divided into two main groups (still A and B) of 6 mice each corresponding to VCO and PKO. Each of the main groups was further divided into 2 sub-groups (4 and 5) of 3 mice and administered with higher doses of 4,000 mg/kg and 5,000 mg/kg of the each oil sample, respectively. After 24 hours, still there was no mortality.

2.2.5 Determination of the Physicochemical Properties of the Oils

2.2.5.1 Determination of Density

The mass of 5 mL of each oil sample was determined with an electronic weighing balance (Mettler Toledo MS-TS, United Kingdom). Exactly 1.0 mL of each sample and distilled water was weighed at room temperature and their respective masses recorded. Density was then calculated with the formula:

$$Density\ = \frac{mass\ of\ oil}{Volume\ of\ oil}$$

2.2.5.2 Determination of Acid Value

The method described by Kumar and coworkers [16] was employed in the determination of the acid value of the oils. Exactly 10.0 g of the oil was weighed into a dried conical flask and 25.0 mL of a solution of equal volume of 95 % ethanol and ether was added to the oil in each case. The mixture was swirled to mix thoroughly. Phenolphthalein indicator was added and the solution was titrated against potassium hydroxide until the colourless solution became pink, signifying the endpoint. The acid value was calculated thus:

$$Acid\ value\ =\ (56.1)V\frac{N}{W}$$

Where V = volume of potassium hydroxide (mL)
N = Normality of the potassium hydroxide
W = weight of the oil sample used (g)

2.2.5.3 Determination of Saponification Value

This was done using the method of Odoom and Edusei [17]. Exactly 2.0 g of the oil was weighed with an electronic balance into a volumetric flask and 25.0 mL of 1.0 N alcoholic potassium hydroxide was added to the oil. The mixture was heated to boil gently for 45 minutes and 1.0 mL phenolphthalein was added which caused the colourless mixture to become pink. The mixture was titrated with 0.5 N hydrochloric acid until the pink colour disappeared. A blank determination was done without the oil. The saponification value was computed using the formula:

$$Saponification\ value\ =\ 56.1\ x\ N\ x\frac{V_2\ -\ V_1}{W}$$

Where N = normality of HCl
V_1= volume of HCl used in the test (mL)
V_2= volume of HCl used in the blank (mL)
W = weight of the oil (g)

2.2.6 Hypoglycaemic Study

2.2.6.1 Induction of Alloxan-Induced Diabetes

Forty albino rats of mixed sexes (150 – 200 g), maintained at standard laboratory conditions were used for the study. After overnight fasting, alloxan monohydrate, 150 mg/kg, was prepared in distilled water and administered intraperitoneally to each of the animals. The alloxan was prepared in batches to be administered to 4 rats per time.

Exactly 20.0 g of glucose was dissolved in 100 mL of distilled water and administered to the animals to combat early phase of hypoglycaemia following administration of alloxan monohydrate. The rats were fed after 1 hour and 72 hours after, the fasting blood glucose (FBG) of each of the animals was taken using a fine test glucometer by obtaining blood from the tail vein of the animals. Albino rats with values >200 mg/dl were considered diabetic [18].

2.2.6.2 Animal Grouping and Administration of PKO, VCO and Metformin

Thirty of the diabetic rats were divided into 6 groups of 5 animals each. The first, second and third groups received metformin, VCO, and PKO only, respectively while the fourth, fifth and sixth groups received VCO + metformin, PKO + metformin and distilled water, respectively. Metformin was prepared in distilled water and administered at a dose of 7.14 mg/kg while VCO and PKO were administered at a dose of 10 mg/kg representing 15 % of the LD50 of the oils. All administrations were done orally using a cannula and the administration was continued once daily for 14 days. All animals were allowed free access to standard diet and water throughout the diurnal period of the study.

2.2.6.3 Blood Collection and Determination of Fasting Blood Glucose Level

The blood glucose level was determined from overnight fasted rats through the blood obtained from their tail veins using Fine Test glucometer. The FBG level values were obtained at 0 hour (baseline), 1 day, 3 days, 5 days, 7 days, 10 days and 14 days.

2.3 Statistical Analysis

Data are reported as the mean ± SEM of triplicate determinations. Statistical analyses were performed using GraphPad Prism version 5.0 for Windows. One-way ANOVA was used to test for variation in pharmacological activity among the treatment groups. Post hoc comparison of means was performed by Turkey's multiple comparison, and $p < 0.05$ was considered to represent a statistically significant difference between test populations [19],[20].

3. RESULTS

3.1 Extraction and Percentage Yield of VCO and PKO

The hot extraction method gave an appreciable oil yield of about 815 mL of VCO. This represented 19.30 % w/w kernel. Similarly, the hot extraction of PKO gave an impressive oil yield of about 680 mL, representing 13.73 % w/w of kernel.

3.2 Acute Toxicity

In the acute oral toxicity study, the LD50 of both VCO and PKO was discovered to be higher than 5,000 mg/kg.

3.3 Physicochemical Analysis of VCO and PKO

Table 3.1: Physicochemical Properties of VCO and PKO

Oil	Density (g/mL)	Saponification Value (mgKOH/g)	Acid Value (mgKOH/g)
VCO	0.834 ± 0.02	267.712 ± 0.07	0.804 ± 0.09
PKO	0.846 ± 0.02	361.377 ± 1.69	11.088 ± 0.09

Table 3.1 shows the result of the physicochemical parameters of VCO and PKO studied.

3.4 Hypoglycaemic study

Table 3.2: Result of effect of concurrent administration of *Cocos nucifera* and *Elaeis guineensis* oils on the hypoglycaemic activity of metformin after 14 days treatment

Treatment Days	Distilled water (10 mL/kg)	Metformin only (7.14mg/kg)	VCO only (10 g/kg)	PKO only (10 g/kg)	VCO (10g/kg) + Met (7.14mg/kg)	PKO (10 g/kg) + Met (7.14mg/kg)
0	249.00 ± 6.71	383.33 ± 7.31	510.66 ± 9.16	509.00 ± 8.21	402.75 ± 7.27	385.50 ± 8.16
1	260.00 ± 6.44	338.00 ± 5.52	358.00 ± 7.43	334.50 ± 9.13	233.75 ± 4.31	291.00 ± 6.44
3	276.50 ± 5.17	162.00 ± 6.33	316.00 ± 7.34	291.00 ± 6.51	186.00 ± 6.12*	201.00 ± 6.14*
5	282.00 ± 6.14	119.50 ± 4.74	284.00 ± 8.91	258.00 ± 7.35	132.00 ± 5.76*	182.00 ± 6.82*
7	290.50 ± 6.26	96.00 ± 4.07	244.00 ± 4.22	196.00 ± 4.04*	93.00 ± 5.09*	157.00 ± 4.52*
10	285.00 ±7.43	94.00 ± 3.12	225.00 ± 5.28	193.00 ± 3.22*	92.00 ± 5.06*	119.50 ± 5.34*
14	281.00 ± 8.18	94.50 ± 3.06	211.50 ± 5.13	190.00 ± 4.11*	90.00 ± 5.77*	107.00 ± 6.11*

* = significant reduction in glucose level relative to negative control at $p<0.05$

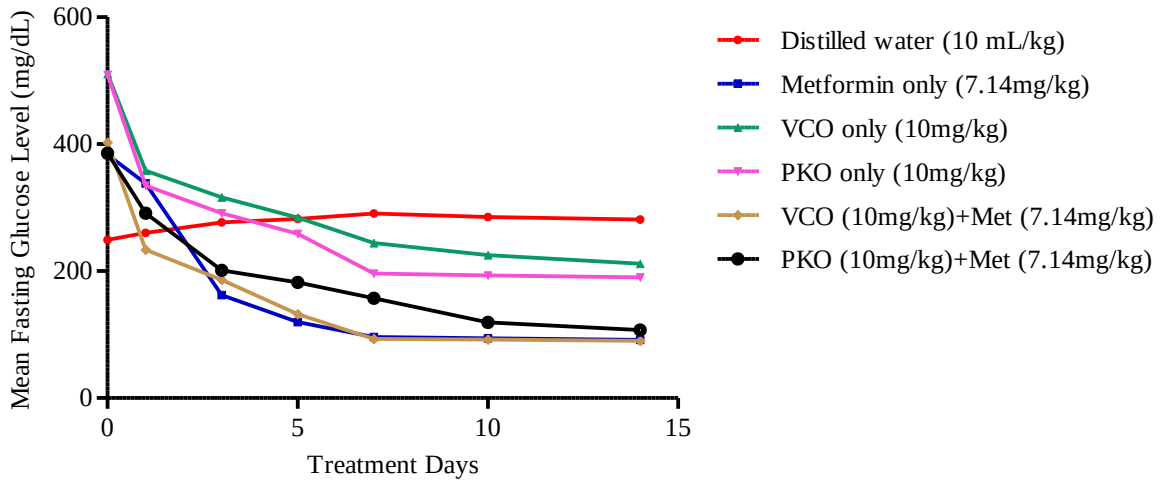

Figure 3.1: Antidiabetic effect of VCO and PKO alone and in combination with metformin on blood glucose levels of alloxan-induced diabetic rat after 14 days treatment.

4. DISCUSSION

The first objective of this study was to extract VCO and PKO. This was done with water to ensure both oils were free from solvent contaminants that may have conflicted with the results of the study. The hot extraction method employed in the extraction of the oils gave a percentage yield of 19.30 % w/w and 13.73 % w/w of kernel of VCO and PKO, respectively. This showed that a gram equivalent of coconut kernel produce more oil than palm kernel. However, the yield is low compared to other methods of extraction, especially the mechanical expression method which has the crude oil yield of palm kernel ranging between 44 % to 53 % w/w [21]. Nevertheless, the yield was dependent on the method of extraction. A similar oil yield value for VCO and PKO were earlier reported by Manikantan [22] and Ayoola [23], respectively. The aqueous hot extraction method has been shown to produce good edible quality oils with better physicochemical properties, higher phytochemical composition and higher antioxidant properties [24]. The high LD50 value of over 5,000 mg/kg of the oils implies that they have very low toxicity and thus very safe for consumption. These advantages made the hot aqueous extractive process suitable for this study. As stated earlier, this method produced relatively small yield of oil compared to mechanical press, chemical and enzymatic methods of extraction which discourages its commercial adoption [25]. The second objective of this study was to analyze the physicochemical properties of the oils (Table 3.1) to give a good insight on their properties with a view to explain their pharmacological properties. On analysis, VCO had a saponification value of 267.71 KOH/g. This value is slightly above the range of 248 – 265 mgKOH/g stated in [26]. However, the value is similar to the saponification value of 269.05 ± 1.6 mgKOH/g reported by [27] for commercial VCO. On the other hand, analysis of PKO revealed that it had a saponification value of 361.38 ± 1.69 mgKOH/g. This value is higher than some values earlier reported [28],[29]. The difference may be due to variation in specie or the degree of rancidity of the oil with respect to the state of the kernel used for the extraction. Saponification value generally is related to the mean molecular weights of the fatty acids making up a fat and inversely related to the chain length of the fatty acid present in the oil. The high saponification values reported in this study shows that the oils possess high content of short and medium chain fatty acids [30]. The physicochemical analysis of the VCO revealed an acid value of 0.804 ± 0.09 mgKOH/g while PKO had a value of 11.088 ± 0.085 mgKOH/g. While the acid value of VCO was lower than 10.0 mg KOH/g which is the standard for named vegetable oils, the acid value of 11.58 mg KOH/g of PKO was slightly above standard [31]. However, the analysed VCO acid value was similar to the earlier reported value [30]. Generally, acid value depends on FFA present in a given oil sample. Studies show that to express acid value in terms of FFA as percent lauric, oleic or palmitic acid, the acid value is divided by 2.81, 1.99 or 2.19, respectively [32]. This relation shows that high levels of FFA content means an increased acid value and vice versa. The acid value is usually used to access the degree of rancidity of samples. The low value in VCO showed that the oil is good and can remain for a fairly long period on shelf without going bad. PKO had a very high acid value but the value was similar to the earlier reported value [28]. The density of VCO was 0.834 g/mL while that of PKO was 0.846 g/mL. These values were similar to most reported values in the literature indicating good quality of the oils [28]. PKO and VCO are highly saturated edible oils but from the physicochemical result, PKO is slightly more saturated than the VCO. The higher the density the more unsaturated the oil [23]. The third objective of the study was to carry out hypoglycaemic study of palm kernel oil and virgin coconut oil in alloxan-induced rats

(Table 3.2). Alloxan induces diabetes through reactive oxygen species (ROS) that leads to a rapid destruction of pancreatic beta cells causing hyperglycaemia [33]. Hyperglycaemia in turn increases the generation of free radicals by glucose auto-oxidation [34]. Results revealed that the daily doses of PKO was able to cause a reduction in mean blood glucose levels compared to negative control, bringing blood glucose levels down to 196 mg/dL (Figure 3.1) which was below the diabetic level within 7 days of administration, although the reduction was not statistically significant at $p < 0.05$ relative to the standard drug. Silalahi [35] in their work reported that PKO reduced blood glucose level from 399 mg/dL to 306 mg/dL within 15 hours showing its hypoglycaemic activity due to its possible antioxidant effect. However, this was not as effective as the standard drug. Although VCO drastically brought down glucose level, it could not bring it down to normal level within 14 days. However, there was no statistically significant difference in the glucose level in the positive control compared to the VCO treatment group. One possible explanation for this could be because of the low content of antioxidants in the oils. Antioxidants are protective agents that inactivate the ROS which cause cell damage [36]. Though not statistically significant compared to the positive control group, VCO however brought down the blood glucose level below the baseline value and kept the level below the baseline. This indicates that VCO possess properties that could possibly control hyperglycaemia in diabetic patients, probably due to its high lauric acid content [37]. Relative to the negative control, there was a statistically significant ($P<0.05$) reduction in glucose level in days 7 – 14 by both PKO. The fourth objective of the study was to assess the effect of concomitant administration of metformin with VCO and PKO on the efficacy of metformin. Metformin, a potent biguanide used orally in small doses in the management of type 2 diabetes mellitus, decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Ultimately, it decreases gluconeogenesis causing hypoglycemia [38], [39]. From the results in Table 3.2 and Figure 3.1, it was observed that the concomitant administration of VCO with metformin showed a very slight improvement on the efficacy of metformin. VCO has been reported to possess antioxidant effects due to its high lauric acid content [37]. This antioxidant effect of VCO on the pancreatic beta cells is possibly responsible for the slight improvement in the efficacy of metformin. VCO possesses additional benefits to diabetics in addition to its glycaemic control; the higher polyphenolic fraction of VCO is suggested to be responsible for its anti-inflammatory and antioxidant effects, which all work toward the prevention of cardiovascular diseases by preventing the progression of atherosclerosis which is one of the complications of diabetes mellitus [40]. The concomitant administration of VCO and metformin may have conferred these additional benefits on the animals while synergistically controlling their glucose level. On the other hand, the concomitant administration of PKO and metformin did not show any improvement on the efficacy of standard drug. Metformin possesses low lipophilicity and consequently, rapid passive diffusion of metformin through cell membranes is unlikely [39]. Concomitant administration of metformin with PKO may possibly have resulted in reduction in the absorption of metformin in the gut due to its low lipohilicity and may possibly result in reduction in the efficacy of metformin in glycemic control due to reduced bioavailability of the drug. Food decreases the extent of and slightly delays the absorption of metformin [41]. Relative to the negative control, the concomitant administration of VCO and PKO with metformin resulted in statistically significant ($P<0.05$) reduction in glucose level in the animals from days 3 – 14. However, the alteration in the hypoglycaemic potential of metformin occasioned by concomitant administration of VCO and PKO was not statistically significant at 95 % confidence level relative to the standard drug.

5. CONCLUSION

The results obtained from the research shows that *Cocos nucifera* and *Elaeis guineensis oils* extracted by hot process retained their physicochemical and pharmacologic activity and possess hypoglycaemic activity. The combination of VCO with metformin resulted in a very slightly synergistic activity of metformin while the concomitant administration of PKO resulted in antagonistic hypoglycaemic effect.

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Conflict of Interest

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Contribution of the Authors

ASE designed the research and wrote the manuscript. VUA, ECJ conducted the physico-chemical analysis and proof read the manuscript; AEU conducted the toxicity study; OAE coordinated the research and vetted the results;

ASU collected the samples and carried out the extraction; EEA and FDI carried out data analysis ; GEC, SSE and DST conducted the hypoglycaemic study. All authors read the manuscript.

REFERENCES

[1] Ighodaro O, Adeosun A, Akinloye O. Alloxan induced diabetes, a common model for evaluating the glycemic control potential of therapeutic compounds and plants extracts in experimental studies. *Medicina* 2017; 53: 365-374.

[2] Sircar NN. Medicinal plants. The Eastern Pharmacist 1982; 29(291): 49-52

[3] Yuan H, Ma Q, Ye L, Piao G. The Traditional Medicine and Modern Medicine from Natural Products. *Molecules* 2016; 21(5): 559-577.

[4] Koparde AA, Doijad RC, Magdum CS. Natural Products in Drug Discovery, In: Perveen S. Al-Taweel A (eds.) Pharmacognosy - Medicinal Plants, 2019. IntechOpen, DOI: 10.5772/intechopen.82860. Available from: https://www.intechopen.com/books/pharmacognosy-medicinal-plants/natural-products-in-drug-discovery.

[5] Maitra A, Abbas AK. Endocrine system. Robbins and Cotran Pathologic basis of disease (7th edn). , Philadelphia: Saunders. 2005, 1156-1226.

[6] Baynest H. Classification, pathology, diagnosis and management of diabetes mellitus. *Journal of Diabetes and metabolism* 2015; 6(5): 2-9.

[7] Seino Y, Nanjo K, Tagima N, Kadowaki T, Kashiwagi A, Araki E, Ito C, Inagaki N, Iwamoto Y, Kasuga M, Hanafusa T, Haneda M, Ueki K. Report of the Committee on the classicication and diagnostic criteria of diabetes mellitus. *Journal of Diabetes Investigation* 2010; 1(5): 212-228.

[8] Sanhueza L, Durruty P, Vargas C, Vignolo P, Elgueta K. Diabetes Mellitus: A Group of Genetic-Based Metabolic Diseases. In: Khan, J, Hsieh, P. (eds.) Cellular Metabolism and Related Disorders, 2019. IntechOpen, DOI: 10.5772/intechopen.89924.
Available from:
https://www.intechopen.com/books/cellular-metabolism-and-related-disorders/diabetes-mellitus-a-group-of-genetic-based-metabolic-disease

[9] Youdim A. Nutrient-drug interactions. Available at: https://www.merckmanuals.com/professional/nutritional-disorders/nutrition-general-considerations/nutrient-drug-interactions.

[10] Maidin N, Ahmad,N. Protective and antidiabetic effects of virgin coconut oil (VCO) on blood glucose concentration in alloxan induced diabetic rats. *International Journal of Pharmacy and Pharmaceutical* Sciences, 2015; 7(10): 57- 60.

[11] Schmittdiel JA, Gopalan A, Lin MW, Berjee S, Chau CV, Adams AS. Population health management for diabetes: Health care system level approaches for improving quality and addressing disparities. *Current Diabetes Rapports* 2017; 17(5): 31.

[12] Agarwal, R. Extraction process of virgin coconut oil. *MOJ Food Processing and Technology*, 2017; 4(2): 54-56.

[13] Ezeoha SL, Akubuo CO, Ani AO. Indigenous design and manufacture of palm kernel oil screw press in Nigeria: problems and prospects. *International Journal of Applied Agricultural Research* 2017; 7(2): 67 – 82.

[14] Nkafaniya I, Maina H, Osemeahon S, Modibbo U. Percentage oil yield and physicochemical properties of different groundnut species (*Arachis hypogaea*). *African Journal of Food Science* 2010; 4(7): 418 – 421.

[15] Lorke D. A New Approach to Practical Acute Toxicity Testing. *Archives of Toxicology*, 1983; 54: 275 – 287.

[16] Kumar G, Lakshmi N, Deena C, Sekhar V, Nikhitha N, Begum H, Bhavani V, Kumar P. Determination of the quality of coconut oils (unrefined grade) and (refined grade) produced from three survey regions of East Godavari District, India. *Asian Journal of Applied Chemical Research* 2018; 2(4):1-8.

[17] Odoom W, Edusei VO. Evaluation of saponification value, iodine value and insoluble impurities in coconut oils from Jomoro District of the Western Region of Ghana. *Asian Journal of Agriculture and Food Sciences* 2015; 3(5): 494 – 499.

[18] Husni A, Purwanti D, Ustadi. Blood glucose level and lipid profile of streptozotocin-induced diabetes rats treated with sodium alginate from *Sargassum crassifolium*. *Journal of Biological Sciences* 2016; 16: 58-64.

[19] GrphPad Prism: User's Guide. Version 5.0 for windows. GraphPad Software, La Jolla California, USA. www.graphpad.com.

[20] Ebong AS, Eseyin OA, Etim EI, Anah VU, Udobre AS, Johnson EC, Charles GE. Effect of *Telfairia Occidentalis* on the pharmacokinetic parameters and efficacy of dihydroartemisinin and amodiaquine combination therapy. *World Journal of Pharmacy and Pharmaceutical Science* 2019; 8(11): 1 – 20.

[21] Ihediwa V, Ndukwu M. Properties, machines and processes for industrial extraction and refining of palm kernel oil: a brief guide. *NIEA conference proceedings* 2017; 38: 131-139.

[22] Manikantan MR, Arivalagan M, Mathew AC, Hebbar KB. Effect of processing parameters on recovery of hot process virgin coconut oil and co-product utilization. *Journal of Plantation Crops* 2015; 43(2): 21 – 27.

[23] Ayoola AA, Anawe PAL, Ojewumi ME, Amaraibi RJ. Composition of the properties of palm oil and palm kernel oil biodiesel in relation to the degree of unsaturation of their oil feedstocks. *International Journal of Applied and natural Sciences* 2017; 5(3): 1 – 8.

[24] Idu M, Ovuakporie-Uvo O, Omoregie ES, Omosigho M. Physicochemical properties, antioxidant activity and phyto-nutritional composition of cold and hot press coconut oils. *GSC Biological and Pharmaceutical Sciences*, 2018; 5(1): 56 – 66.

[25] Rosenthal A, Pyle DI, Niranjan K. Aqueous and enzymatic processes for edible oil extraction. *Enzyme and Microbial Technology* 1996; 19(6): 402 – 420.

[26] NAFDAC, 2019. National Agency for Food and Drug Administration and Control. Fats and oils regulations, 1-24. Available at: https://www.nafdac.gov.ng/wp-content/uploads/Files/Resources/Regulations/New_Draft_Regulations/Fats-and-oils-Reguations-2019-clean.pdf

[27] Prapun R, Cheetangde N, Udomranti S. Characterization of virgin coconut oil (VCO) recovered by different techniques and fruit maturities. *International Food Research Journal* 2016; 23(5): 2117-2124.

[28] Atasie V. Akinhami T. Extraction, compositional studies and physico-chemical characteristics of palm kernel oil. *Pakistan Journal of Nutrition* 2009; 8(6): 880-803.

[29] Amira O, Babalola O. Oyediran M. Physicochemical properties of palm kernel oil. *Current Research Journal of Biological Sciences* 2014; 6(5): 205 – 207.

[30] Ghani N, Chanip A, Hwa P, Ja'afar F, Yasin H. Usman A. Physicochemical properties, antioxidant capacities, and metal contents of virgin coconut oil produced by wet and dry processes. *Food Science and Nutrition 2018;* 6(5): 1298-1306.

[31] Codex Stan – Food and Agriculture Organisation of the United Nations. Available at: www.fao.org/fao-who-codexalimentarius/en/

[32] AOCS Official Method Cd 3d-63 – Sampling and analysis of commercial fats and oils; Acid value, Reapproved 2009. Available at: www.global.ihs.com/doc_detail.cfm?document_name=AOCS%20CD%203D%2D63&item_s_key=00572831

[33] Stanley P, Prince M, Menon VP. Hypoglycaemic and other related actions of *Tinospora cordifolia* roots in alloxan-induced diabetic rats. *Journal of Ethnopharmacology* 2000; 70(1): 9-15.

[34] Bajaj S, Khan A. Antioxidants and diabetes. *Indian Journal Endocrinology and Metabolism*, 2012; 16(2): 267–271.

[35] Silalahi J, Rosidah PE, Satria D. Hypoglycaemikc effect of hydrolysed palm kernel oil in rats. *Der Pharma Chemica*,2016; 8(20): 182-186.

[36] Joseph SA, Theophilus FC, Dominic EA, Rita DA, Kwaw MA, Kofi A, Eric W, George KA, Alfred AA, Henry BD. Antihyperglycaemic and antioxidant effects of *Adenia lobata* Engl. (Passifloraceae) in streptozotocin-induced diabetic rats. *African. Journal of Traditional, Complementary, and Alternative Medicine* 2013; 10(5): 386–393.

[37] Iranloye B, Oludare G, Olubiyi M. Anti-diabetic and antioxidant effects of virgin coconut oil in alloxan induced diabetic male Sprague Dawley rats. *Journal of Diabetes Mellitus 2013;* 3(4): 221-226.

[38] Kirpichnikov D, Mcfalane S, Sower J. Metformin: An update. *Annals of International Medicine*, 2002; 137(1): 25-33.

[39] Graham R, Hardie DG. Pearson ER. The mechanisms of action of metformin. *Diabetologia*, 2017; 60(9): 1577–1585.

[40] Babu A, Veluswamy S, Arena R, Guazzi M, Larvie C. Virgin coconut oil and its potential cardioprotective effects. *Postgraduate Medicine, 2014;* 126(7): 76-83.

[41] Dumitrescu R, Mehedintu C, Briceag I, Purcarea V, Hudita D. Metformin-clinical pharmacology in PCOs. *Journal of Medicine and Life* 2015; 8(2): 187-192.