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Effect of N-Hexane Oil Extract of Two Spices on Serum Lipid Profile and Blood Glucose Concentration of Albino Rats

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Abstract - Consumers are concerned with their health and physical fitness and are seeking for alternative plant products with potential for providing nutrients with enhanced health benefits. Hence, this study investigates the effect of mixture of Ehuru (*Monodora myristica*) and Njasang (*Ricinodendron heudelotii*) oil extract on serum lipid profile and blood glucose concentration of albino rats. The spices were processed into fine flour and the oil was extracted with n-hexane as the solvent. A total of twenty five rats weighing 125-160g were separated into five groups of five each to represent control, olive oil and varying concentrations of the spices. After acclimatization for one week, experimental administration of the extract was carried out daily for 28 days. Blood samples were collected by cardiac puncture into tubes. A portion of the blood was used for fasting blood glucose determination. Serum was separated from the other portion and used for assay of lipid profile using standard kit methods. The results obtained showed percentage fatty acid yield for Ehuru and Njasang as 79.54 and 81.0 (polyunsaturated) and 13.40 and 15.0 (monounsaturated) respectively. Fasting blood glucose assay showed that only rats in group 1 (6.46mmol/L) became significantly ($p < 0.05$) hyperglycaemic while groups 2-4 (6.03, 5.98 and 5.53mmol/L) showed a hypoglycaemic effect with respect to control (6.13mmol/L). Serum total cholesterol and low density lipoprotein-cholesterol were significantly ($P < 0.05$) low for test samples and olive oil compared to control. The serum high density lipoprotein-cholesterol (1.15 – 1.55mmol/L) increased with increase in extract administration compared to control (1.02mmol/L). Triglyceride level also increased (0.60-0.65mmol/L) for test samples compared to control (0.6mmol/L), but the increase was not significant at $P < 0.05$.

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1. INTRODUCTION

The need to maintain good health is the driving force in the search for alternative oil seeds of spices with high medicinal and nutritional potentials. Spices, depending on the part of the plant being used can be classified into fruits, seed, leaves or floral parts and bulbs used to season food due to their distinctive flavor and aroma (Manay, 1987), as well as for therapeutic purposes (Sirinivasan and Sambaiah, 1991). Herbs and spices are integral part of the daily diet. Dietary spices influence various systems in the

body such as gastrointestinal, cardiovascular, reproductive and nervous systems resulting in diverse metabolic and physiological actions (Angerer et. al., 2002). There is an increasing demand for edible oils with essential fatty acids. Achinewhu et al; 1995; investigated chemical composition of thirty wild spices indigenous to Nigeria and observed that they contained high amount of fats as well as essential oils.

Research have been able to show a relationship between edible oils and cardiovascular disease. It has been reported severally that oils with high amounts of cholesterol and other saturated fatty acids tend to cause hyperlipidaemia (accumulation of lipid in the walls of the arteries) causing a narrowing of the blood vessels and an attendant effect on blood pressure (Guthrie and Picciano, 1995). Data from experimental and epidemiological studies show that elevated levels of Low density lipoprotein (LDL) – cholesterol and Triglycerides and low levels of High density lipoprotein (HDL) - cholesterol are major risk factors of coronary heart disease (Besong et. al., 2011). It has also been shown that glucose and fatty acids appear to interact in health and disease as they work together to regulate the expression of several enzymes involved in carbohydrate metabolism thereby affecting blood glucose levels. Ugochukwu et. al., 2003 reported that high levels of triglycerols and total serum cholesterols often accompanying diabetic condition were significantly decreased by the ethanolic extract of *G. latifolium*. Spices like cinnamon, cloves, bay leave and turmeric have insulin-potentiating effect in vitro (Khan et. al., 1990). So, these spices might have a role in lipid metabolism. Seed oil from spices has been reported as good sources of essential oil. However, no oil from a single source can be suitable for all purposes. Spices also, are rarely consumed singly, they are consumed either as mixture of two or more spices to improve their quality. It is, therefore, important to understand the influence of such combination using n-hexane oil extract of ehuru (*Monodora myristica*) and Njasang (*Ricinodendron heudelotii*) oil seeds. Literature on their therapeutic properties in vivo is scanty, hence, this present study is considering the effect of the oil extract mixture of both spices on serum lipid profile and blood glucose concentration of albino rat.

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II. MATERIALS AND METHODS

a) Plant Materials

The seeds of ehuru (*Monodora myristica*) and njasang (*Ricinodendron heudelotii*) were purchased from the herb sellers at mile 3 market, Port Harcourt, Rivers State Nigeria. The seeds were cleaned, ground and then sieved to 300mm mesh. The sieved spices were then stored in an air tight container and stored in desiccators for subsequent analysis.

b) Animals

Twenty five (25) albino rats of both sexes weighing 125-160g were obtained from the animal house of the Department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria. They were housed in cages of five groups of five each and acclimatized for one week with food and water ad libitum at room temperature (26 - 28°C) throughout the 28 days of the experiment. The standard laboratory animal care was followed in this study (CIMOS, 1985).

c) Oil Extraction from Ehuru and Njasang

Soxhlet extraction was carried out using n-hexane. Fifty (50) gram of ground seeds were placed in a cellulose paper and extraction lasted for 8 hours. The oil was then recovered by evaporating the solvent using rotary evaporator (Bligh and Dyer 2009). Fatty acid composition of the seed oil of the spices was determined using NUCON series 5700 gas chromatography equipped with the flame ionization detector and stainless steel packed column, having internal diameter 2mm and length 2.0cm. About 0.1ml of oil was converted to the methyl ester by using the borontrifluoride and extracted in 1ml hexane before being injected into the Gas Chromatography (GC). The detector temperature was programmed for 200°C with a flow rate of 25ml/min. The injector temperature was set at 200°C. Column temperature was programmed from 70°C to 200°C with the increasing rate of temperature 6°C/min. Nitrogen was used as the carrier gas. Hydrogen flow 40ml/min and air flow 60ml/min were used. The peak was identified by measuring the retention time of the samples and comparing it with the standard under the same operating conditions.

d) Experimental Procedure

Rats were administered with the mixture (prepared in equal proportion) of the oil extract orally at 0.5% per kg body weight in doses of olive oil: test sample (1:3, 1:2 and 1:1) for groups, 1, 2 and 3 respectively. Whereas group 4 was administered with 2.0ml olive oil and group 5 which served as control received 0.9% oral normal saline solution. All animals were observed daily. After 28 days of treatment blood samples were collected by direct cardiac puncture. The blood was transferred into a sterilized sample container and a portion was used for fasting blood glucose assay;

while, the other portion was allowed to stand at ambient temperature and immediately after clotting, it was centrifuged at 2000G for 5 min. the sera were carefully separated into a clean container and kept in the refrigerator at 4°C and analyzed within 24hr.

e) Glucose Assay

Fasting blood glucose concentrations were measured by using the glucose oxidase method with an automated glucose analyzer (Roche Diagnostic GmbH, Mannheim, Germany)

f) Lipid Profile

Serum lipid profile which includes high density lipoprotein (HDL) -cholesterol, total cholesterol (TC) triglycerides (TG) and low density lipoprotein (LDL) - cholesterol were determined by standard enzymatic colorimetric techniques which involves test kits procured from Randox Laboratories.

g) Weight Measurement

Body weights of the rats were measured three times in a week for 28 days. Daily changes in body weights as percentages were recorded. The percentage of daily changes in the body weights was calculated according to the following formula.

Change in body weights (%) =

$$\frac{100 \times (\text{weight}_{\text{final}} - \text{weight}_{\text{initial}})}{\text{Weight}_{\text{initial}}}$$

h) Statistical Analysis

Results were reported as mean $\pm$ SD. All data were analyzed using the analysis of variance. When analysis of variance revealed a significant effect, means were separated using Duncan's new multiple range test (Wahua, 1999).

III. RESULTS

a) Fatty acid Profile

The fatty acid composition of Ehuru and Njasang spices is summarized in Table 1. Ehuru and Njasang showed the following fatty acid percentages. 79.54 and 81.0% for polyunsaturated fatty acids, 13.40 and 15.0 for monounsaturated fatty acids and 7.10 and 5.50 for saturated fatty acids respectively.

Table 1 : Fatty acid composition of Ehuru and Njasang

Fatty acid	No. of carbon	Oil seed (%)	
		Ehuru	Njasang
Caprylic	8.0	1.0	-
Capric	10.0	0.55	1.90
Lauric	12.0	1.40	1.20
Myristic	14.0	1.60	2.30
Palmitic	16.0	5.42	ND
Stearic	18.0	2.02	ND
Myristoleic	14.1	5.30	14.74
Palmitoleic	16.1	5.42	ND
Oleic	18.1	2.44	ND
Linoleic	18.2	34.48	26.76
Linolenic	18.3	41.45	32.20
α -linolenic	18.3	2.20	ND
Eicosapentaenoic	20.5	ND	20.60
Total fat		38.72	43.20

ND $\Rightarrow$ Not Detected

b) *Effect of oil seed spices on blood glucose*

The result on the effect of oil extract of Ehuru and Njasang on blood glucose is shown in Fig. 1. Only rats in group 1 (6.40mmol/L) became significantly ($P<0.05$) hyperglycaemic with respect to the control group. The hyperglycaemic effect was dose-dependent. There was no significant ($P<0.05$) difference with rats in groups 2, 3 and 4 compared to control.

c) *Effects of oil seed spices on lipid profile*

Table 2 shows the general serum lipid profile after administering the oil extract of the spices to the rats

for 28 days. Total cholesterol (TC), LDL-cholesterol, and LDL/HDL - cholesterol ratio values were low for test samples (group 1-3) and olive oil (group 4) compared to control. The decrease was significant ($P<0.05$) for all samples. HDL-cholesterol (1.15-1.55mmol/L) increased with increase in extract administration. The increase observed in HDL was significant ($P<0.05$). Triglyceride (TG), slightly increased for test samples compared to control, but the increase was not significant at $P<0.05$.

Table 2 : Serum Lipid Profile

Group	Lipid Profile (mmol/L)				
	TC	TG	LDL	HDL	LDL/HDL
1 (1:3)	2.14 ^b	0.65 ^a	0.48 ^b	1.55 ^a	0.31 ^c
2 (1:2)	2.12 ^{ab}	0.62 ^b	0.44 ^c	1.36 ^b	0.32 ^c
3 (1:1)	2.08 ^c	0.60 ^b	0.43 ^c	1.20 ^c	0.36 ^b
4 (olive oil)	1.95 ^d	0.58 ^c	0.40 ^d	1.15 ^d	0.35 ^b
5 (control)	2.20 ^a	0.60 ^b	0.58 ^a	1.02 ^e	0.97 ^a

Values are expressed as mean $\pm$ SD Means in the columns not followed by the same superscripts differ significantly ($P\leq 0.05$).

d) *Body weight changes*

The rats gained weight throughout the experimental period as shown in Fig. 2. The weight increase in groups 4, 3 and 2 were significant ($P\leq 0.05$) and very steady compared to groups 1 and 5.

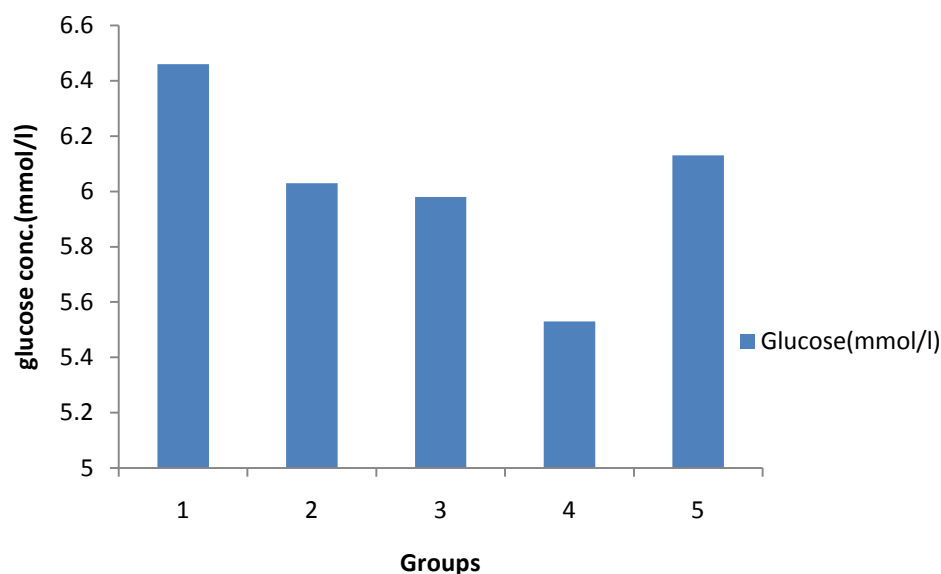


Fig. 1 : Glucose conc.(mmol/l) in the various groups

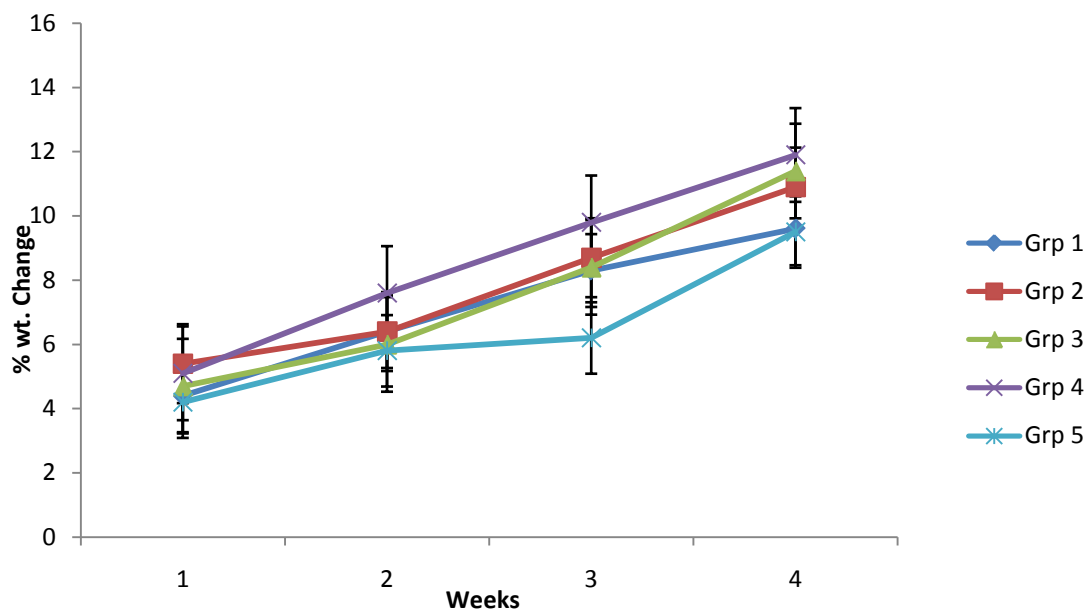


Fig. 2 : Weekly body weight changes in the rats

IV. DISCUSSION

Table 1 reported the fatty acid composition of Ehuru and Njasang. The relative abundance of mono and polyunsaturated fatty acids present in these samples reveals their potentials in nutrition with enhanced health benefit. Linoleic, oleic and linolenic acids present in these extract are fatty acids that have a cholesterol lowering effect which prevents coronary heart disease and arteriosclerosis (Colussi et. al., 2007). Linoleic acid as well as its derivatives serves as structural components of the plasma membrane and as precursors to regulate specific cellular metabolic functions and gene expression (Jump, 2002). The fatty acid composition of these samples is also similar to

other vegetable oils such as canola oil, olive oil and sun flower oil (Matos et. al., 2009).

Data from this present study show that n-hexane oil extracts of mixed spices exert hypoglycaemic effect as well as hyperglycaemic effect at higher concentration. Chang and Johnson 1980, reported similar results. The mechanism by which spices lower plasma glucose and the interaction of high fat diets on glucose metabolism has not been fully elucidated (Khan et. al., 1990). Although fatty acids are said to be structural components of membrane lipids such as glycolipids. High lipid levels have been associated with increase in serum glucose level which is as a result of insulin inhibition (Raza and Movahed, 2003; Harvey and

Champe, 1994). However some plants like cinnamon, ginger, bay leaves and garlic have been reported to have beneficial effects in the treatment of both diabetes and cardiovascular diseases (Khan et. al., 2003). Ugwuja et al; 2008 also reported similar result using equal proportions of mixture of curry, garlic and ginger.

Variation in the amount of dietary fat has been reported to affects plasma cholesterol as well as coronary heart diseases. Dietary saturated fatty acid increase the total blood cholesterol level as dietary polyunsaturated fatty acids decrease it. (Guthrie and Picanno, 1995; Sardesai, 1992; Norum, 1992). The oil seed extract of mixture of ehuru and njasang lowers the serum total cholesterol (TC) and low density lipoprotein (LDL) - cholesterol. The observed decrease is probably due to high percentage of polyunsaturated fatty acids and monounsaturated fatty acid present in the samples. Although, saturated fatty acids raise the level of LDL. The mechanisms involved are not completely understood, however the receptor on liver cell membrane that binds LDL cholesterol appears to be suppressed by saturated fatty acids. For example, when LDL receptor activity decreases by the presence of saturated fatty acids, LDL catabolism decreases and blood level of LDL increase and vice versa (Guthrie and Picciano 1995; Fraser, 1994). The insignificant increase in Tryglyceride (TG) observed in all the groups may be attributed to the presence of saturated fatty acids especially in Ehuru sample. It has also been reported that sometimes there may be a defective effect on the enzyme (lipase) that degrades triglyceride (Enig, 1993). Besides, Norum 1992 reported that individual saturated fatty acids differ in their ability to change serum lipid. The serum HDL-cholesterol level significantly increased. This indicates that these mixtures can promote decreased risk of coronary heart disease (Colussi et. al., 2007). Also, the LDL/HDL cholesterol ratio decreased significantly. This ratio is thought to be the atherogenic index of lipoprotein (Ajayi et. al., 2011) compare to an increase in serum total cholesterol level which is said to be associated with increased risk of atherosclerosis. The lower the LDL/HDL ratio the less atherogenic the lipoprotein profile is thought to be (Murray et. al., 2003). The hypolipidaemic and hypoglycaemic effect observed in this study agree with the reports of Khan et al, 2003; Gorinsterin et al; 2006; Ugwuja et. al., 2010. However, at higher concentration slight hyperglycaemic and hyperlipidaemic effect was observed. While group 4 treated with Olive oil showed a lowered glucose and serum lipid levels.

The body weight gain observed in this study may be attributed to the nutrient potentials of these spices, some of which include the stimulatory effect on the digestive system. Platei and Srinivasan, 1996, reported that ginger enhances the digestive activities of enzymes like the intestinal lipase, sucrase and maltase.

V. CONCLUSION

Observations made from this study prove that mixture of oil extract of Ehuru and Njasang at culinary dose has beneficial health effect on blood glucose and lipids. The combination of spices as is currently practiced is then encouraged.

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