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Comparative Effects of Two Edible Vegetable Oils in South East Nigeria on Dexamethasone Induced Dyslipidaemic Albino Wistar Rats

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Abstract

Dyslipidaemia is a risk factor for cardiovascular diseases. A lipid profile of an organism is a direct measure of three blood components namely; total cholesterol (TC), triglycerides and high density lipoproteins cholesterol (HDL-C). Hypercholesterolemia is a lipoprotein metabolic disorder characterized by high serum low density lipoprotein and blood cholesterol and one of the most important risk factors for the development of cardiovascular diseases and lipid abnormalities. There have been many claims that most, if not all brands of vegetable oil in Nigeria is cholesterol free. Hence the study was undertaken to see the effect of vegetable oils in Nigeria on the lipid profile of albino rats induced with dyslipidaemia using dexamethasone. The phytochemical analysis and lipid profiles of albino rats treated with two vegetable oils were undertaken. Five groups of five rats were used in the study as follows: Group 1: Normal control- no induction no treatment, Group 2: Induction of dyslipidemia using dexamethasone (1 mg/kg bw) for 5 days only, Group 3: Treatment of induced dyslipidemia using a standard statin (20 mg/kg bw), Group 4: Induction + 3 ml/kg bw vegetable oil after induction, Group 5: Treatment without dyslipidemia using vegetable oil (3 ml/kg bw). The treatment lasted for 14 days and thereafter, the rats were fasted overnight and blood samples were taken through ocular puncture. The results showed various phytochemicals such as terpenoids, steroids, flavonoids, phenolics, tannins and alkaloids. The lipid profiles of the albino rats revealed that a non-significant ($P>0.05$) increase was observed in serum total cholesterol level of groups 3 and 5 when compared to group 2 while group 4 recorded a non-significant ($P>0.05$) decrease when compared to group 2. There was a non-significant ($P>0.05$) increase in serum triacylglycerol level of groups 3, 4, and 5 when compared to group 2. The result also showed a non-significant ($P>0.05$) decrease in serum HDL level in groups 3, 4, and 5 compared to group 2. Also, group 3 indicated a significant ($P<0.05$) increase in serum LDL level compared to group 2 while group 4 recorded a non-significant ($P>0.05$) decrease in serum LDL level compared to group 2. However, a significant ($P<0.05$) increase in serum LDL level was recorded in group 5 compared to group 2. The second vegetable oil revealed that group 3 indicated a non-significant ($p>0.05$) increase in total cholesterol level while groups 4 and 5 recorded a significant ($p<0.05$) decrease compared to group 2. Groups 3, 4 and 5 recorded a non-significant ($p>0.05$) increase in TAG compared to group 2. Group 3 recorded a significant ($p<0.05$) increase in LDL compared to group 2. Group 4 recorded a non-significant ($p>0.05$) decrease while group 5 recorded a significant ($p<0.05$) decrease in LDL compared to group 2. Groups 3, and 5 recorded a non-significant ($p>0.05$) decrease in HDL compared to group 2. Group 4 showed a significant ($P<0.05$) decrease in HDL compared to group 2. However, among the oil treated groups, TAG was found to be within range compared to the control group. Thus, the results of this present study imply that the consumption of vegetable oil for a long time could influence the risk of cardiovascular disease since they elicited an elevation of LDL-cholesterol and lowered HDL-cholesterol. Caution should, therefore, be applied on the consumption of vegetable oils as continuous use may have impact on lipid profile thereby causing dyslipidaemia.

Keywords: Dyslidaemia, vegetable oil, lipid profile dexamethasone, rats.

INTRODUCTION

Lipids are compounds that do not dissolve in water appreciably, but are rather soluble in organic solvents such as chloroform, ether, hexane among others. Lipids are made up of different elements. They are carbon, hydrogen and oxygen atoms, and in some instances contain phosphorus, nitrogen, sulphur and other elements depending on the site and function. Fats and oils are the main components of lipids present in foods (Oresic, 2009). The role of lipids cannot be over emphasised. Lipids are present in many types and forms of cells. They participate in the generation of acetyl Co A which ultimately passes through the tricarboxylic acid cycle and the electron transport chain for ample ATP generation. They are also implicated in transcription of genes, regulation of vital metabolic pathways and physiological responses (Gurr *et al.,* 2016). Lipids are of different seful in the overall development and wellbeing of different organisms (Simopoulos, 2011). Some lipids act as second messengers and regulators of gene expressions (Zuniga *et al.,* 2011). Cholesterol, another type of lipids, is a precursor molecule for the synthesis of steroid hormones (Dawson *et al.,* 2009). Lipids are implicated in the aetiology of some ailments (Valenzuela and Videla, 2011). This, by extension affects the lipid requirements in humans (Katan *et al.,* 1994). Lipids play crucial role in providing sensory characteristics that enhance general acceptability of foods.

A lipid profile of an organism is a function of its cholesterol (TC), triglycerides and high density lipoproteins cholesterol (HDL-C) (Dinsmoor, 2006). High cholesterol levels in the blood is predominantly known for subsequent high serum low density lipoprotein and serum and posses the challenge of the development of cardiovascular diseases (CVD) and dyslipidaemia (Matos *et al.,* 2005). The challenge of CVD is alarmingly progressive and the chunks of those affected are in developing countries as posited by Beaglehole and Yach, 2003. Dietary habits, sedentary or mobile lifestyle, attitude of care-givers towards diabetes mellitus and smoking habits may affect lipid profile (Attia *et al.,* 2015). Treatment of dyslipidaemia entails reducing significantly outrageous quantity of low density lipoprotein. (Grundy *et al.,* 2004). There is no evidence to suggest that there is a threshold level of total cholesterol (TC) below which there is a reduced risk of developing chronic heart disease (CHD) as reported by Chen *et al.,* 1991. The combination of these CVD risk factors does increase cardiovascular disease, that causes sickness and death (World Health Organization, 2002). However, dyslipidemia has been recognized as a strong and independent risk factor for CVD (Kadiri, 1999). The statistics of gerantological atherosclerosis point to the fact that age may be implicated as a factor that affects lipid metabolism; by so doing doing aged people are disposed to having CVD (Marhoum, 2013). It was estimated in 2002 that, 29% of death world-wide (16.7 million deaths) were due to CVD (World Health Organization, 2011).

Dexamethasone is a glucocorticoid medication used to treat different ailments such as rheumatic problems, asthma, chronic obstructive lung disease a number of skin diseases, severe allergies, brain swelling, among others (Kritchevsky *et al.,* 2003).

There have been many claims that most, if not all brands of vegetable oil in Nigeria is cholesterol free. Hyperlipidemia is one of the recognized risk factors for cardiovascular disease (CVD). Different reports have established the correlation between high lipid levels and the development of CVD (Downs *et al.,* 1998). Behrman and Venkat (2005) reported that five percent cholesterol is present in vegetable oils. Hence, this study was to investigate the effect of some vegetable oils on lipid profile parameters of dexamethasone-induced dyslipidemia in Wistar albino rats with the view to validating or otherwise disproving the claims of different vegetable oil manufactures in Nigeria.

MATERIALS AND METHODS

The seeds of *chrysanthus albidum* were purchased in a local market in Nsukka Enugu State Nigeria. Conical flasks (pyrex, England), Water bath (Gallenkamp, England), Beakers (pyrex, England), Weighing balance (Metler HAS, U.S.A), Filter papers (Whatman), Test tubes (pyrex, England), Measuring cylinder (pyrex, England), Glass funnel (pyrex, England), Spectrophotometer (Spectronic 20D, Germany), Centrifuge (Vickas Ltd, England) and Rotary evaporator.

Chemicals

Dexamethasone and Randox Biochemical diagnostic kits were used in the study. All other kits and reagents used in this study were of analytical grade.

Quantitative Phytochemical Analyses

The phytochemicals which are present in the vegetable oils were determined and quantified by standard procedures.

Determination of Total Phenolic Compounds:

Folin-Ciocalteu reagent 1.5 ml was added in 100 µl of aqueous ethanol extract of the samples. 20% of Na_2CO_3 solution was added and the samples were incubated in the dark at room temperature for 15 minutes after which the absorbance was taken at 765 nm. These data were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of Gallic acid.

Determination of Concentration of Alkaloids

The method described by Harborne, (1973) was used to determine the amount of alkaloids present in the extract. A quantity 0.2 g of the sample was weighed into a container and 2 ml of 20% sulphuric acid and 2 ml ethanol were added and mixed with 1ml of 60% H_2SO_4. Five minutes later 1 ml of 0.5% formaldehyde in 60% H_2SO_4 was added and the solution was allowed to stand

for 3 hours. The absorbance of the sample was taken at 565 nm.

Determination of Concentration of Tannins

The method described by Harborne, (1973) was used to determine the amount of total tannins present in the extract. A quantity of 0.2 g of the sample was weighed into a sample container. Aqeous ethanol was used to marcerate the sample. 0.5 ml of the supernatant was transferred to triplicate tubes. 0.5 ml Folin-Ciocalteau's reagent. Five minutes later 0.5 ml of 2% Na2CO3 was added followed by addition of 4 ml distilled water. The solution was incubated for 30 minutes, centrifuged for 10 minutes at 3000 rpm and the supernatant was read at 725 nm.

Determination of Concentration of Flavonoids

Total flavonoids content of King's oil was determined by the aluminum chloride colorimetric assay. A 0.2g of sample was weighed and marcerated with 6 ml ethyl acetate. The supernatant was shaken with 6 ml aqueous ammonia for five minutes. The supernatant was discard and the infra-natant was read at 490nm. The flavonoid contents were determined using standard curve generated from quercetin.

Determination of Concentrations of Steroids.

The Steroids contents of the vegetable oils were determined. 0.2 g of the sample was weighed and marcerated with 5 ml ethanol. The supernatant (0.8 ml) was mixed with 0.8ml of chromogen and 0.3 ml concentrated H_2SO_4 and allowed to stand for 30 minutes. The absorbance was taken at 550 nm.

Animals

Twenty-Five Wistar rats of weight average 150 and 180g were purchased from animal house of Shalom Laboratories Nsukka. The rats were housed in the animal house of the laboratory. The rats were kept in cages at room temperature. They were fed with poultry mash (Finisher) and had free access to water.

Experimental Design

Dyslipidaemia was induced by intra-peritoneal injection of dexamethasone (1mg/kg bw) for 5 consecutive days. Dyslipidaemia was confirmed by determining the serum lipid profile before the commencement of treatment with the samples and standard drug- statin. Blood samples were collected through ocular puncture. The animals were divided into five groups as follows:

Group 1: Normal control- no induction no treatment
Group 2: Induction of dyslipidemia using dexamethasone (1 mg/kg bw) for 5 days only
Group 3: Treatment of induced dyslipidemia using a standard statin (20 mg/kg bw)
Group 4: Induction + 3 ml/kg bw vegetable oil after induction
Group 5: Treatment without dyslipidemia using vegetable oil (3 ml/kg bw)

The treatment lasted for 14 days and thereafter, the rats were fasted overnight and blood samples were taken through ocular puncture. The blood samples were allowed to clot and centrifuged at 3000 rpm for 10 minutes and the serum obtained was used for biochemical analyses.

Lipid Profile Assay

Total cholesterol, triacylglycerides , low density lipoprotein cholesterol, and High density lipoprotein cholesterol (HDL-C) levels were estimated using assay kits (Randox UK) according to the manufacturer's instructions.

Statistical Analysis

All data were expressed as mean ± SD. The mean values of the data were statistically analyzed using one-way analysis of variance (ANOVA) using SPSS version 21. The significant differences among the groups were further analyzed by Duncan's Multiple Range Test. The P values less than 0.05 were considered as statistically significant.

Quantitative Analysis of Phytochemicals in the known Vegetable Oils.

Table 1 shows the result of the quantitative phytochemical constituents of the known vegetable oils. The result showed the presence of the following phytochemicals of flavonoids (3.27 ± 0.42), (A only) terpenoids (1.58 ± 0.64 and 0.99±0.46), steroids (0.30 ± 0.01and 0.07±0.02), and tannins (0.12 ± 0.01 and 0.17±0.04). Among the secondary metabolites present flavonoids content was highest (3.27 ± 0.42%) and terpenoids (1.58 ± 0.64%) in sample A. However, total phenolics and alkaloids were not detected in both samples.

Table 1: Quantitative Analyses of Phytochemical compositions of the known Vegetable Oils

Phytochemicals Amount ± SD (mg/100g) A	B
Terpenoids 1.58 ± 0.64	Terpenoids 0.99±0.46
Steroids 0.30 ± 0.01	Steroids 0.07±0.02
Flavonoids 3.27 ± 0.42	Flavonoids ND
Tannins 0.12 ± 0.01	Tannins 0.17±0.04
Total Phenolics ND	Total phenolics ND
Alkaloids ND	Alkaloids ND

Values are presented as mean ± SD

A. Effects of the known Vegetable Oils on Lipid Profile on dexamethasone-induced dyslipidemia in Wistar Albino Rats

Table 2A showed a non-significant (P>0.05) increase in serum total cholesterol level in groups 2, 3, 4, and 5 compared to group 1. A non-significant (P>0.05) increase was observed in serum total cholesterol level of groups 3 and 5 when compared to group 2 while group 4 recorded a non significant (P>0.05) decrease when compared to group 2. Meanwhile, groups 4 and 5 revealed a non-significant (P>0.05) decrease in serum total cholesterol level compared to group 3. However, group 5 recorded a non-significant (P>0.05) increase in serum total cholesterol level compared to group 4. Table 2 showed a non-significant (P>0.05) decrease in serum triacylglycerol level of groups 2, 4, and 5 compared to control group 1 while a non-significant (P>0.05) difference was observed in serum triacylglycerol level of group 3 compared to group 1. But, groups 3, 4, and 5 recorded a non-significant (P>0.05) increase in serum triacylglycerol level when compared to group 2. However, a non-significant (P>0.05) decrease in serum triacylglycerol level was seen in group 4 compared to group 3 while group 5 recorded a non-significant (P>0.05) increase in serum triacylglycerol level compared to group 3. Meanwhile, group 5 recorded a non-significant (P>0.05) increase in serum triacylglycerol level compared to group 4. Table 2 indicated a non-significant (P>0.05) increase in serum high density lipoprotein (HDL) level of groups 2 and 3 compared to group 1 while a non-significant (P>0.05) decrease in serum HDL level of groups 3 and 5 was observed compared to group 1. Also a non-significant (P>0.05) decrease in serum HDL level was recorded in group 3, 4, and 5 compared to group 2. However, group 4 recorded a non-significant (P>0.05) increase in serum HDL level compared to group 3 while group5 registered a non-significant (P>0.05) decrease in serum HDL level compared to group 3. Perhaps, group 5 showed a non-significant (P>0.05) decrease in serum HDL level compared to group 4. Table 2 recorded a non-significant (P>0.05) decrease in serum Low density lipoprotein (LDL) level of groups 2 and 4 compared to group 1 while group 3 revealed a non-significant (P>0.05) increase in serum Low density lipoprotein (LDL) level compared to group 1. Meanwhile, a significant (P<0.05) increase in serum LDL level was recorded in group 5 compared to group 1. Also, group 3 indicated a significant (P<0.05) increase in serum LDL level compared to group 2 while group 4 recorded anon-significant (P>0.05) decrease in serum LDL level compared to group 2. However, a significant (P<0.05) increase in serum LDL level was recorded in group 5 compared to group 2. Meanwhile, group 4 recorded a significant (P<0.05) decrease in serum LDL level compared to group 3 while group 5 showed a non-significant (P>0.05) increase in serum LDL level compared to group 3. But a significant (P<0.05) increase in serum LDL level was recorded in group 5 compared to group 4.

Table 2A: Effects of a known Vegetable Oil on Lipid Profile on dexamethasone-induced dyslipidemia in Wistar Albino Rats

Groups	TChol. mmol/L	TAG mmol/L	HDL mmol/L	LDL mmol/L
One	$5.80 \pm 0.53^{a,b,c}$	1.83 ± 0.06^{a}	$2.23 \pm 0.76^{a,b}$	$3.20 \pm 0.92^{a,b}$
Two	$6.17 \pm 0.75^{b,c}$	1.67 ± 0.40^{a}	2.73 ± 0.71^{b}	$2.67 \pm 0.90^{a,c}$
Three	7.17 ± 0.72^{c}	1.83 ± 0.06^{a}	$2.10 \pm 0.36^{a,b}$	$4.04 \pm 0.43^{b,d}$
Four	$5.87 \pm 1.89^{a,b,c}$	1.80 ± 0.10^{a}	$2.33 \pm 0.06^{a,b}$	$2.27 \pm 0.57^{a,c}$
Five	$6.57 \pm 0.55^{b,c}$	1.83 ± 00.12^{a}	1.67 ± 0.25^{a}	4.53 ± 0.39^{d}

Values with different superscripts were significant at p<0.05 while those with the same superscripts were non significant at p>0.05.

Key

Group 1: normal control after induction

Group 2: Induction of dyslipidemia using dexamethasone (1mg/kg bw) for 5days only

Group 3: Treatment of induced dyslipidemia using std Statin (20mg/kg bw)

Group 4: Induction + 3ml/kg bw vegetable oil after induction

Group 5: Treatment without dyslipidemia using vegetable oil (3ml/kg bw)

B. Effects of a known Vegetable Oil on Lipid Profile of dexamethasone-induced dyslipidemia in Wistar Albino Rats

The result showed a non-significant (p>0.05) increase in serum total cholesterol level in group 2 and 3 while group 4 and 5 recorded a non-significant (p>0.05) decrease compared to group 1. Group3 indicated a non-significant (p>0.05) increase while groups 4 and 5 recorded a significant (p<0.05) decrease compared to group 2. Groups 4 and 5 recorded a significant (p<0.05) decrease compared to group 3. However, group 5 recorded a non-significant (p>0.05) increase compared to group 4. The result in TAG concentration recorded a non-significant (p>0.05) increase in groups 2 and 4. Group 3 recorded a non-significant (p>0.05) difference while group5 showed a non-significant (p>0.05) increase compared to group 1. Groups 3, 4 and 5 recorded a non-significant (p>0.05) increase compared to group 2. Group 4 recorded a non-significant (p>0.05) decrease while group 5 recorded a non-significant (p>0.05) increase compared to group 3. Group 5 recorded a non – significant (p>0.05) increase compared to group 4. The result in LDL concentration recorded a non-significant (p>0.05) decreases in groups 2 and 4 while group 3 recorded a significant (p<0.05) increase, group5 has a

significant ($p<0.05$) decrease compared to group1. Group 3 recorded a significant ($p<0.05$) increase compared to group 2. Group 4 recorded a non-significant ($p>0.05$) decrease while group 5 recorded a significant ($p<0.05$) decrease compared to group 2. Group 4 recorded a non-significant ($p>0.05$) decrease while gorup5 a significant ($p<0.05$) decrease compared to group 3. Group 5 recorded a non-significant ($p>0.05$) decrease compared to group 4. The result in HDL concentration recorded a non-significant ($p>0.05$) increase in groups 2 and 5 while groups 3 and 4 recorded a non-significant ($p>0.05$) decreases compared to group 1. groups 3, 4 and 5 recorded a non-significant ($p>0.05$) decrease compared to group 2. Group 4 recorded a non significant ($p>0.05$) decrease while group 5 recorded a non-significant ($p<0.05$) increase compared to group 3. Group 5 recorded a non-significant ($p<0.05$) increase compared to group 4.

Table 2B: Effects of a known Vegetable Oil on Lipid Profile on dexamethasone-induced dyslipidemia in Wistar Albino Rats

S/N	Tchol Mmol/L (Mean ±std)	TAG Mmol/L (Mean±std)	LDL Mmol/L (Mean ±std)	HDL Mmol/L (Mean±std)
1	$5.80 \pm 0.53^{a,b,c}$	1.83 ± 0.06^{a}	$3.20\pm0.92^{a,b}$	$2.23\pm0.76^{b,c}$
2	$6.17\pm0.75^{b,c}$	1.67 ± 0.40^{a}	2.67 ± 0.90^{b}	$2.73\pm0.71^{c,d}$
3	7.17 ± 0.72^{c}	1.83 ± 0.06^{a}	$4.04\pm0.43^{a,b}$	$2.10\pm 0.36^{b,c}$
4	3.67 ± 2.90^{a}	1.70 ± 0.30^{a}	$2.89\pm0.78^{a,b}$	1.87 ± 0.38^{a}
5	4.70 ± 0.00^{a}	1.87 ± 0.06^{a}	$1.79\pm0.32^{a,b}$	$2.53\pm0.31^{b,c}$

Values with different superscripts were significant at $p<0.05$ while those with the same superscripts were non significant at $p>0.05$.

Keys

Group 1: normal control after induction

Group 2: Induction of dyslipidemia using dexamethasone (1mg/kg bw) for 5days only

Group 3: Treatment of induced dyslipidemia using std Statin (20mg/kg bw)

Group 4: Induction + 3 ml/kg bw vegetable oil after induction

Group 5: Treatment without dyslipidemia using vegetable oil (3ml/kg bw).

DISCUSSION AND CONCLUSION

Discussion

Globally stroke is on the increase. Generally humans are what they eat and what their genetic make ups are. That means nature and nurture play a central role in different diseases that challenge humanity today. Dyslipidaemia is implicated in stroke and other lipid related diseases. Lipids play different roles in metabolism. Such roles include, but not limited to energy provision, storage of triglycerides and hormone precursors. Investigating the effect of reasonable consumption of vegetable oil on the lipid profile of albino rats will go a long way in striking a possible correlation between this class of food and dyslipidaemic challenges among human populace.

Dexamethasone increases the concentration of plasma free fatty acid, total triglyceride, and VLDL protein, triglyceride, phospholipid, and free cholesterol. Dexamethasone induces elevation of serum lipid profile and consequently may lead to the accumulation of lipids in the liver. Hence, in this study induction of dyslipidemia using dexamethasone (1mg/kg bw) for 5days showed an increased level in Total cholesterol (Tchol) and lowered High density lipoprotein (HDL).

The hypolipidemic effects of statin against dexamethasone suggest its ability to regulate cholesterol biosynthesis and reverse hyperlipidemia by the restoration of cholesterol homeostasis, possibly through inhibition of the action of HMG-CoA reductase along cholesterol biosynthetic pathways (Shukla *et al.,* 2004). However, this study showed that statin caused an inhibition in TAG level as it showed a non-significant ($p>0.05$) difference and a non-significant ($p>0.05$) difference in HDL compared to the control group. This study showed an increased level of lipid profile (TC, TAG and LDL) in the vegetable oil treated groups. Though the vegetable oil increased the total cholesterol level, it effectively decreased the HDL cholesterol too. The results of the phytochemical analyses showed that Flavonoids were more in quantity than the other phytochemicals tested. Flavonoids, including flavones, flavanols and condensed tannins, are plant secondary metabolites, the antioxidant activity of which depends on the presence of free OH groups. Plant flavonoids have antioxidant activity *in vitro* and also act as antioxidants *in vivo* (Geetha, 2003) though at moderate quantity. Quantitative analysis showed that one of the vegetable oil contains higher amounts of flavonoids. From the findings of the study it may be suggested that high amount of flavonoids might have acted as a pro-oxidant and this might have possibly contributed to the dyslipidaemic condition observed in the study.

Conclusion

In some of the vegetable oil treated groups, an increase in LDL with a decrease in HDL was observed. However, among the oil treated groups, TAG was found to be within range compared to the control group. Thus, the results of this present study imply that the consumption of vegetable oil for a long time could influence the risk of cardiovascular disease since they elicited an elevation of LDL-cholesterol and lowered

HDL-cholesterol. Caution should, therefore, be applied on the consumption of vegetable oils as continuous use may have impact on lipid profile thereby causing dyslipidaemia.

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