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Alterations in lipid profile of normal male Wistar rats administered aqueous extracts of Phoenix dactylifera, Cyperus esculentus, soybean, and liquid cod-liver oil

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Abstract:

This research study was designed to determine changes in lipid profile of normal male wistar rats subjected to daily oral administration of 1000mg/kg phoenix dactylifera, 1600mg/kg Cyperus Esculentus, 1000mg/kg soybean aqueous extracts and 0.5ml liquid cod liver oil for five weeks. The lipids profile analyzed in this study includes total serum cholesterol, triglyceride, high density, low density, and very low density lipoproteins using enzymatic point millimoles and precipitant methods measured in mill moles per liter. Other parameters examined are weight measurement in grams and fasting blood sugar level in millimoles per liter respectively. **Results:** The result from this study shows an increase in serum total cholesterol (4.10mmol/l), triglyceride (1.71mmol/l), HDL (1.78mmol/l) and low density lipoprotein (3.09mmol/l) compared with the control group. There was an increase in LDL among the test groups except in group 4 that received 1000mg/kg extract of soybean having a decrease of 2.08mmol/l compared with control (2.22mmol/l). A non-significant decrease in very low density lipoprotein was also observed among the test groups except in grop2 (0.73mmol/l) compared with the control (0.70mmol/l). More findings from this study revealed a very strong positive correlation between serum total cholesterol versus other lipoproteins and between HDL versus other lipid parameters examined. Further observation from this study indicate a decrease in weight among the test groups at the end of the 5th week of the study compared with week one and the control. The FBS level was significantly decreased among the entire test group that received the aqueous extract of Cyperus Esculentus and phoenix dactylifera. **Conclusion:** The aqueous extract of phoenix dactylifera and Cyperus Esculentus significantly and effectively reduces the weight, fasting blood sugar and triglyceride levels with an inverse increase in the concentration of good cholesterol (HDL) needed for the maintenance of cardiovascular health status among study groups.

Keywords:

Cholesterol, Lipoproteins, Cod-liver oil, Lipids, Phoenix dactylifera, Cyperus Esculentus, soybean.

INTRODUCTION

These are combination of soluble proteins needed for lipid transportation in blood plasma. Lipoproteins composition and classes include very low density lipoprotein, high density lipoprotein, low density, chylomicrons and intermediate density lipoproteins. The chylomicrons are particles of large triglyceride synthesize by the intestine and are responsible for the transportation of dietary cholesterol to peripheral tissues and the liver. Triglyceride removal from chylomicron in peripheral tissues by an enzyme lipoprotein lipase decreases its level to chylomicron remnant that has pro-artherogenic properties (Kenneth and Feingold, 2024; Beyersmann, 2008).).Elevated lipid profile such as total cholesterol and the low density lipoproteins in blood plasma with a decrease in high density lipoproteins are among the risk factors leading to the development of cardiovascular diseases worldwide.

Serum lipid profile is modified factors that are sensitive to obesity and prostate hypertrophy. Previous studies have shown that decrease in high density lipoprotein accompanied by increase in low density and triglyceride is strong predictors to tumor grades. High density lipoprotein transport cholesterol from body cell to the liver with anti-inflammatory and antioxidant role that may decrease the progression of prostate cancer. Increased level of triglyceride is major factors contributing to the development of prostate cancers by increasing oxidative stress including insulin resistance (Panelniva & Ossie, 2019).

Dietary supplements and drugs that promote high density lipoproteins and decrease LDL as well as cholesterol and triglycerides will help to reduce the growing prevalence of cardiovascular diseases associated with increase mortality (Onyeali *et al.*, 2010; Alfred T (2002).An increase in total cholesterol levels in the blood result due to high regular intake of fatty diets. This is true because in fat metabolism the fats consumed are converted to cholesterol while fat derivatives from local food is transported to the liver but the occurrence of elevated cholesterol level is established during disturbance in the formation of cholesterol within the liver and small intestine. An elevated LDL will thus trigger plaque formation within the intima of blood vessels leading to arteriosclerosis formation (Isddiyanto *et al.*, 2020).Cod liver oil are destearinated oil of fresh livers from codus morrhua L, cadidae and other species. They are rich sources of vitamin A, omega3-fatty acid, D &E essential for inflammatory reduction, strong bones formation etc (Achuba, (2005); Marriam Webster, 2023).It can be taken as dietary supplement for the improvement of sight anomalies (macular degeneration) and for the promotion of good health to both brain and coronary arteries as potent antioxidants (Simon *et al.*, 2915; Vetter *et al.*, 2013).

MATERIALS AND METHODS

The fruits and seeds were purchased in Kano state of Nigeria while the analysis of the extracts was done in the pharmaceutical laboratory department of the University of Port Harcourt, Nigeria using methods of experimental procedures adopted by the association of official analytical chemist of 2016.

Study Design: The grouping and dosage of extract administered daily per body weight are as follows:

All the animals in the test group were given the extract orally ones daily at different milligrams per body weight as shown below:

Group 1 (control) received feeds and water ad libitum

Group 2 received 0.5ml of 1600mg/kg Cyperus Esculentus extract

Group 3 received 0.5 of 100mg/kg phoenix dactylifera extract daily

Group 4 received of 1000mg/kg soybean aqueous extract daily

Group 5 received 0.3ml of 800mg/kg of Cyperus Esculentus and phoenix dactylifera aqueous extract respectively

Group 6 received 0.2ml of Cyperus Esculentus, phoenix dactylifera and soybean extract combined

Group 7 received 0.3ml of 500mg/kg standard drug-liquid cod liver oil daily.

Acclimatization: The animals were all weighed using Golden Meter USA weighing scale calibrated in grams following the expiration following their acclimatization at the end of fourteen days and housed in mesh wire cage under normal temperature, twelve hours light and night darkness for five weeks at the University of Port Harcourt. The declaration of Helsinki regarding the principle of experimental care was strictly followed.

Experimental animals: Forty nine (49) healthy male wistar rats weighing between 97.25g to 232.00g were randomly selected for this study and were housed in the pre-clinical laboratory for research studies.

LIPID PROFILE DETERMINATION

Cholesterol (Enzymatic and-point method)

- Principle: The cholesterol was determined following enzymatic hydrolysis andoxidation. Quinoneimineas indicator was formed from hydrogen peroxide and4-aminoantipyrine in the presence of phenol and peroxidase.
- Procedure: Label the tubes as test, standard and blank.
- Pipette 1.0ml of the reagent into all the tubes
- Add 10ul of the standard samples and d/w into appropriate tubes.
- Mix and incubate for 10mins at25'c.
- Read and record the absorbance at540nm

Unit mmol/l

Triglycerides (mmol/L)

- Principle: The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine is formed from hydrogen-peroxide, 4 – aminophenazone and 4 – chlorophenol under the catalytic influence of peroxidase.
- Procedure: Label the tubes as test, standard and blank
- Pipette 1.0ml of the reagent into all the tubes
- Add 10ul of standard, sample & d/w into appropriate tubes: Mix and Incubate for 10mins at 25'c
- Read and record the absorbance at540nm.

HDL – Cholesterol (Precipitant Method) Unit mmol/L

- Principle: Low density lipoprotein, VLDL and Chylomicron fractions are precipitated by the addition of phosphotungestic acid in the presence of magnesiumions. After centrifugation the cholesterol concentration in the HDL fraction which remains in the supernatant is determined.

- Procedure: Labeled the tubes as std, blank and test.
- Pipette 0.7m of the rgt into all the tubes.
- Add 40ul of the std, sample and d/w into respective tubes
- Mix and centrifuge 10mins at4000rpm
- Label the tubes as, test std and blank
- Pipette 1.0ml of Chol (rgt) into all the tubes.
- Add 0.1ml of the supernatant into appropriate tubes
- Mix and incubate for 10mins at25'c.
- Read and record the absorbance at540nm

Fasting Blood Sugar Determination: The fasting blood sugar level was measured using an ACCU-CHEK glucometer machine (mannhe in Germany) with serial number GB 21383167/ ref 06993761001 CE0123 and test strips (code 333) were used in the morning hours before feeds and extract was administered. Fresh capillary blood was introduced into the test point of each strip inserted into the glucometer after a sterilized lancet was used to prick the tail and the results displayed on the screen.

RESULTS

Table 1: Changes in the Mean Values of Lipid Profile

Parameters	Grp1	Grp2	Grp3	Grp4	Grp5	Grp6	Grp7	p-value
TC (mmol/L)	3.25±0.92	3.50±0.99	4.10±0.14	3.10±0.14	3.25±0.64	3.50±0.28	3.55±0.21	0.71
TG (mmol/L)	1.55±0.49	1.61±0.42	1.71±0.27	1.20±0.14	1.34±0.30	1.51±0.19	1.38±0.25	0.71
HDL (mmol/L)	1.73±0.24	1.51±0.33	1.78±0.49	1.66±0.24	1.74±0.30	1.76±0.33	1.66±0.26	0.05
LDL (mmol/L)	2.22±0.90	2.71±0.84	3.09±0.31	2.08±0.16	2.22±0.46	2.52±0.42	2.51±0.56	0.54
VLDL (mmol/L)	0.70±0.21	0.73±0.18	0.68±0.01	0.55±0.06	0.61±0.13	0.69±0.09	0.67±0.16	0.55

Key's: TC=Total Cholesterol, TG=Triglyceride, HDL=High density lipoprotein, LDL= Low density lipoprotein, VLDL= Very low density lipoprotein. Note: p-values of 0.05and below are considered significant.

Table 2: Correlation between TC and other Lipid Profile

Variables	R-values	p-value
TC versus TG	0.863**	0.00
TC versus HDL	0.753**	0.02
TC versus LDL	0.952**	0.00
TC versus VLDL	0.779**	0.01

Note: **Correlation is significant at 0.01 and 0.05 level (2-tailed).

Table 3: Correlation between HDL Versus other parameters

Variables	R-values	p-value
HDL versus TG	0.654**	0.01
HDL versus LDL	0.534**	0.04
HDL versus VLDL	0.681**	0.00
TG versus LDL	0.885**	0.00
LDL versus VLDL	0.763**	0.00

A strong positive correlation exist between triglyceride and HDL, TG and LDL, TG and VLDL. A strong significant positive correlation were also identified between HDL versus VLDL and LDL as well.

Table 4: Weight Evaluation between Control and Test Groups

Weight(g)	Grp1 (control)	Grp2	Grp3	Grp4	Grp5	Grp6	Grp7
Week 1	97.25±66.83	134.00±5.48	151.25±8.77	142.25±97.07	187.50±17.07	178.00±116.00	232.00±37.10
Week 5	103.10±72.21	115.17±27.62	145.27±10.01	145.10±102.06	115.02±130.00	163.25±109.05	178.77±122.17
p-value	0.00	0.03	0.03	0.08	0.00	0.04	0.00

Table 5: Initial week and Final week FBS (mmol/l) levels Compared

Groups	1 (Control)	2		3	4	5	6	7	p-value
Week 1	4.24±1.45	4.22±0.35		3.60±0.56	4.14±0.51	4.33±0.50	3.87±0.54	3.27±1.27	0.05
Week 5	4.16±0.24	3.08±0.12		3.28±0.44	4.00±0.26	4.05±0.42	3.35±0.21	4.60±0.04	0.04

DISCUSSION

An elevated serum lipid profile is among the contributing factors leading to the development of cardiovascular diseases increasing in its frequencies among population in developed countries (Uvoh *et al.*, 2017). Lipids are fatty oily compound that are soluble in organic solvent but insoluble in water that forms part of the cell membrane which control the movement of substances in and out of the cell. They are also responsible in the formation of hormones, storage of energy and absorbing fat soluble vitamins such as ADEK. Cholesterol are synthesize from dietary saturated fats by the liver (Bronner *et al.*, 1993).

Results: The result from this investigative study shows a decrease serum total cholesterol (3.10mmol/l), triglyceride (1.20mmol/l).high density lipoprotein (1.66mmol/l),low density lipoprotein (2.08mmol/l) and very low density lipoprotein (0.55mmol/l) in group4 that received 1000mg/kg aqueous extract of soybean daily compared with the control group of 3.25,1.55,1.73, 2.22 and 0.70mmol/l lipid profile. There was a non-significant increase in group2 and 3 serum total cholesterol (3.50 and 4.10mmol/l), triglycerides (1.61 and 1.71mmol/l), low density lipoprotein (2.71 and

3.09mmol/l) and very low density lipoprotein (0.73mmol/l) that received 1000mg/kg body weight of extract of phoenix dactylifera daily compared with the control. However we noticed a decrease in high density lipoprotein in group2, group4 & 7, though group3 treated at 1000mg/kg phoenix dactylifera extract and the rest had a significant increase in HDL compared with the control.

A non-significant increase of low density lipoprotein known as bad cholesterol was observed in group2, 3, 6 and 7 compared with the control group. Similar increase in lipid profile of treated wistar rats have been earlier reported by Malik and sabahelkhier (2019).However Solomon *et al*, 2023 reported a decrease in the so called bad cholesterol (LDL) among test groups compared with the control group from their research studies on lipid profile of male wistar rats. Furthermore the very low density lipoprotein outcome from the present study were all lower among the test groups with the exception of group2 only that has a slight increase compared with the control. There was a significant positive correlation between total cholesterol versus other lipoproteins and high density lipoprotein versus other lipid profile. Normal reference values for cholesterol and triglycerides in wistar rats range between41-126mg/dl (2.27mml/l-7mmol/l)and30-409mg/dl (UCLADLAMRev, 2013).

Weight: There was a significant p value increase in the final weight (103.19g) in the control group compared with their initial weight (97.25g).This final increase was contrary to the test groups that had a significant decrease in their final weight compared with their initial weight except in group4 having moderate increase with no significant p-value. The increase in the HDL level (1.78mmol/l) compared with control (1.73mmol/l in group3 is of physiological importance since this could lead to the prevention of plaques formation and arteriosclerosis within the intima of blood vessels and maintain a healthy cardiovascular status.

FBS: The fasting blood sugar level decreases significantly among group2-6 during the fifth week of the study compared with the first week after twelve hours food abstinence. However we observed an increase in group7 final (5^{th}) week fasting blood sugar level that received liquid cod liver oil compared with week one and other groups. The body breaks down food into glucose after a meal to release energy for the maintenance of a possible close range of sugar level within the blood. The goal of maintaining sugar normalcy is to prevent the risk caused by diabetes and help the body feel healthy (James *et al.*, 2022).

Conclusion: Findings from this study indicate decrease in triglycerides, fasting blood sugar levels and weight among the study groups with an inverse increase in good cholesterol (HDL) level. The outcome from this study is a useful tool for reducing the risk associated with hyperlipidemia.

Conflict of interest: There is no conflict of interest among the authors.

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