Effect of Ethanol Extract of *Cucurbita pepo* Leaves on the Lipid Profile of Wistar Albino Rats

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Authors’ contributions

This work was carried out in collaboration between all authors. Author FUE designed the study. Author GCU performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors FUE, CMO and GCU managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

ABSTRACT

Aim: The role of nutrition in lipid metabolism continues to generate a lot of research interest, especially as dyslipidaemia is implicated in a host of diseases. The work investigated the effect of ethanol extract of *Cucurbita pepo* leaves on the lipid profile of Wister rats.

Methodology: A total of 18 rats divided into three groups of 6 rats each were employed in the investigation. The first group (baseline) was sacrificed after purchase; second group (control) was fed rat chow, and the third group (test) was fed a composite feed containing rat chow and 10% extract of *C. pepo* leaves.

Results: The lipid profile (total cholesterol, triglycerides, LDL, HDL and VLDL) of the rats was assayed after 18 days of feeding and the result showed no significant difference ($P>0.05$) between the test group and control for the lipids assayed. However, there was a marked increase in the HDL level of the test group (0.500±0.057 mmol/L) compared to the control (0.268±0.043 mmol/L), it was nonetheless statistically non-significant ($P=0.068$). The HDL increasing effect observed may stem from the rich phytochemistry of the leaves, as

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preliminary phytochemical investigation showed the leaves to contain saponin, tannin, alkaloids, flavonoids and glycosides. **Conclusion:** We conclude that the leaves of *C. pepo* may play a medicinal role in maintaining lipid homeostasis.

**Keywords:** *Cucurbita pepo*; leaves; lipid profile; hypolipidemia; wistar rats; ethanol extracts.

**1. INTRODUCTION**

Lipids are naturally occurring organic substances, which are soluble in non-polar solvents but insoluble in polar solvents. They are the principal form of energy in most organisms and the major constituent of cellular membrane. Triacylglycerols, steroids, phospholipids and glycolipids are examples of lipids. As evidenced by several research endeavors, aberrant lipid metabolism is linked with a host of diseases including type 2 diabetes mellitus, obstructive sleep apnea, coronary artery disease, nonalcoholic fatty liver diseases and cancer [1,2]. Significant elevation or depression of specific lipid types has been shown to correlate strongly with metabolic disorders in several clinical studies, and hence estimation of specific lipids—total cholesterol, triacylglycerol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL)—is typically used in accessing lipid disorders [3–5].

The implication of lipid dysfunction in health and disease has informed a large number of research in the field of lipid metabolism, especially as affected by diet and nutrition. As a corollary, the effect of various dietary components on serum lipid profile and metabolism has informed several clinical investigations [6,7].

*Cucurbita pepo* L, is a cultivated plant of the genus (cucurbita) which includes varieties of squash, gourd and pumpkin. It is cultivated in warmer climates, and serves and food in many countries of the world [8]. Parts of *C. pepo* are of ethnomedical importance, and have been empirically demonstrated to have therapeutic benefits; including inhibition of prostatic hyperplasia, anti-ulcer activities, wound healing potential, hypoglycemic effect [8–10].

In the present work, the effect of diet-supplementation with the extract of *C. pepo* leaves on lipid profile of rats was studied to observe for hypolipidemic potential. Previous works in the area have paid mind to the hypolipidemic potential of the fruits and seeds [11–13]. Hence, this work is novel in the sense that it pays attention to the leaves, which hitherto, had received very little attention.

**2. MATERIALS AND METHODS**

**2.1 Sample Collection and Preparation**

The fresh leaves of pumpkin (*Cucurbita pepo*) used in this work were bought from a local market in Nsukka, Enugu State, Nigeria and was authenticated by a taxonomist in the Department of Botany, Nnamdi Azikiwe University Awka, Nigeria.

The leaves were washed with distilled water and dried under room temperature for two weeks. Thereafter, the leaves were ground into fine powder using a manual blender. The obtained powder was macerated in 70% ethanol for 24 h, after which the solution was filtered and the ethanol evaporated using a Soxhlet apparatus. The obtained filtrate (extract) was used to supplement standard rat chow to obtain the experimental feed.

**2.2 Experimental Feed Formulation**

The leaf extract of *C. pepo* and standard rat chow were mixed in a ratio of 1: 10 to obtain the experimental diet. The composition of the pelletized commercial rat chow (Vital Grower’s Feed) had a proximate composition of; crude protein (13%), fat (8%), crude fibre (15%), calcium (0.9%), phosphorous (0.35%), energy (2600 Kcal/kg).

**2.3 Animal Handling and Grouping**

A total of 18 male Wistar albino rats (aged 8-13 weeks), purchased from the Animal farm of Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, were used in the experiment. The animals were handled following standard ethical guidelines on animal handling and research. The rats were divided into three groups (Group 1-3) of 6 rats each. The first group (Group 1) served as a baseline; they were sacrificed shortly after purchase and had their blood collected via cardiac puncture. The blood
samples were centrifuged and the obtained sera used for biochemical investigations. The baseline gives the biochemical status of the rats prior to use in the experiment, as experimental conditions and ambience where animals are kept combine to affect biochemical indices. Group 2 (test group) received the experimental feed made of 10% of the leaf extract of C. pepo and 90% of grower's mash for 18 days. The final cohort (Group 3) received only the grower's mash for 18 days and served as the experimental control. Both groups were also given water ad libitum.

2.4 Sample Collection for Biochemical Investigation

After 18 days of feeding the animals (G2 & 3), they were fasted for 12 h, were weighed and thereafter anaesthetized using chloroform. Blood samples were collected from the rats via cardiac puncture. The obtained samples were centrifuged at 1000 rpm for 30 min to obtain the sera, which was employed for the lipid profile analysis.

2.5 Lipid Profile Investigation

2.5.1 Estimation of serum total cholesterol

The cholesterol content of the serum was measured at 546 nm using Auto-chemistry analyser (Mindray BA-88, China). The cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator, quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.

2.5.2 Estimation of serum triacylglycerol

The Triglyceride content of the serum was measured at 546 nm using Auto-chemistry Analyser (Mindray BA-88). The triglycerides (GPO) method is based on the enzymatic determination of glycerol using the enzyme glycerol phosphate oxidase (GPO) after hydrolysis by lipoprotein lipase. The principle of this method was described by Fossati [14] who coupled the reaction with the classical Trinder reaction sequence. The reaction quantitates the total glycerides in serum including the mono and diglycerides, and the free glycerol fractions.

2.5.3 Estimation of serum low-density lipoprotein (LDL)

Again, this was analyzed using the Auto-chemistry Analyzer (Mindray BA-88) using the auto LDLTM Cholesterol Reagent. This was determined by measuring the amount of cholesterol remaining in the serum after precipitation with polyvinyl sulphate. The LDL content was estimated as the difference between the total cholesterol and the cholesterol remaining in the solution after precipitation.

2.5.4 Estimation of serum high-density lipoprotein (HDL)

This was determined using the Auto-chemistry Analyzer (Mindray BA-88) using the auto HDLTM Cholesterol Reagent. This was determined by measuring the amount of cholesterol remaining in the serum after precipitation of LDL, VLDL and Chylomicron by the addition of phosphotungstic acid and magnesium chloride. The HDL content was measured as the remaining cholesterol in the sample solution after precipitation.

2.5.5 Estimation of serum very low-density lipoprotein

This was calculated using the Friedewald's method [15] for calculation very low-density lipoprotein.

\[ \text{VLDL-c} = \frac{\text{Triglycerides}}{5} \]

2.6 Qualitative Phytochemical Analysis of Cucurbita pepo

Alkaloids: One ml of the extract was mixed with 5 mL of 2% HCl in a test tube, heated on water bath, and filtered. Of the filtrate, 2 mL was divided into two aliquots of 1 ml each. To the first portion, few drops of Wagner's reagent were added; the occurrence of reddish-brown precipitate is taken as a positive test. To the second aliquot, 1 ml of Mayer's reagent was added and the appearance of buff-coloured precipitate will be an indication for the presence of alkaloids [16].

2.6.1 Flavonoids

Lead acetate test: To 1 ml of the filtrate, 1 ml of 10 % lead acetate solution was added. The appearance of a buff-coloured precipitate indicates the presence of flavonoids [17].

Ferric Chloride test: To 1 mL of extract in a test tube, a few drops of 10% FeCl2 was added. A green-blue or violet coloration indicates the presence of a phenolic hydroxyl group [17].

Saponins: The extract (1 ml) was boiled with 5 ml of distilled water, the soluble fraction of the
mixture was decanted into two aliquots while still hot. The obtained filtrate was used for the following tests:

**Emulsion test:** Two drops of olive oil was added to 1 ml of the extract in test tube. The set up was mechanically agitated and observed for formation of an emulsion; which indicates the presence of saponin.

**Frothing test:** Distilled water (3 mL) was added to 1 ml of the extract. 0.5 mL filtrate was diluted to 5 mL with distilled water and shaken vigorously for 2 minutes. Formation of stable froth head indicates the presence of saponin [18].

**Reducing Sugars:** To 2 mL of the extract, a few drops of Fehling’s solution A and B were added; an orange-red precipitate suggests the presence of reducing sugar.

**Cyanogenic glycosides:** Two ml of the extract was dispensed into a 100 mL conical flask containing 10 ml of water. Using a thread, a picrate paper was suspended into the flask and the flask’s bore covered with a cork. The set-up was heated in a water bath for 1 h. Change in colour of the paper from yellow to brick red indicates the presence of cyanogenic glycosides.

**Protein:** To 5 ml of distilled water was added to 0.1 g of the ground sample and left for 3 min and then filtered. To 2 ml portion of the filtrate, 0.1ml of millions reagent was added, shaken and kept for observation. A yellow precipitate indicates the presence of protein.

**Carbohydrate:** A quantity, 0.1 g, of the ground sample was shaken vigorously with water and then filtered. Few drops of molisch reagent were added to the aqueous filtrate followed by vigorous shaking. Then 1ml of concentrated sulphuric acid was carefully added to form a layer below the aqueous solution. Formation of a brown ring at the interface indicates the presence of carbohydrate.

**Tannins:** A quantity, 2 g of the ground leaves was boiled with 5 ml of 45% of ethanol for 5 minutes. The mixture was cooled and filtered. The filtrate was used for the following tests:

i. **Lead sub-acetate:** To 1 ml of the filtrate, 3 drops of lead sub-acetate solution was added. A gelatinous precipitate indicates the presence of tannins.

ii. **Bromine Water:** To 0.1 ml of the filtrate, 0.5 ml of bromine water was added and then observed for a pale brown precipitate.

iii. **Ferric Chloride:** 1 ml of the filtrate was diluted with distilled water and 2 drops of ferric chloride were added. A transient greenish to black colour indicates the presence of tannins.

**2.7 Statistical Analysis**

All statistical analyses were performed using SPSS 23.0 (SPSS, Inc., Chicago, USA). In order to determine the variance between means of the different groups, one-way ANOVA tests were performed, followed by post-hoc test (Tukey HSD) to compare means. A P value < .05 was considered significant. Data are presented as mean ± standard deviation (SD).

**3. RESULTS AND DISCUSSION**

The role of nutrition in lipid metabolism continues to generate a lot of interest, and the result of this work further highlights how diet can affect the milieu of serum lipid. For total cholesterol and triglycerides, there was a significant increase in both the test and control groups compared to the baseline group. This finding may suggest that the rat was fed fat-lean diet in the farm they were

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean concentration of lipid ± sd (mmol/L)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Tot. Chol</td>
</tr>
<tr>
<td>Baseline</td>
<td>0.28±0.23a</td>
</tr>
<tr>
<td>Control</td>
<td>0.87±0.16a</td>
</tr>
<tr>
<td>Test</td>
<td>1.06±0.01b</td>
</tr>
</tbody>
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Table 1 above showing the mean values (mmol/L) for total cholesterol (Tot. chol), triglycerides, low density lipoprotein (LDL), high density lipoprotein (HDL), and very low density lipoproteins (VLDL) in the three groups of rat. Values with same superscript (a, b) differ significantly (P< 0.05).
originally purchased from. However, despite the marked elevation of total cholesterol and triglycerides in the control and test groups compared to the baseline, they had cholesterol and triglyceride concentrations within the optimal range (6.29 mmol/L and 4.22 mmol/L, respectively) observed in Wister rats of comparable age and sex [19]. Also, there was no significant difference in the levels of the two lipid between the test group (fed leave extract of *C. pepo*) and the control (fed only commercial rat chow), this may suggest that the leaves of *C. pepo* may not have any significant hypercholesterolemic or hypertriglyceridemic effects. In fact, several studies on the seed and seed-oil of *C. pepo* suggest it may have lipid lowering potential [11,12].

There is robust evidence associating marked elevation of LDL-cholesterol with several pathological conditions, including cardiovascular diseases (CAD), diabetes and inflammation [20]. In the current work, there was no significant elevation of LDL in the rats fed the experimental diet compared to the control or baseline group, further reinforcing the beneficial attribute of the leaves of *C. pepo*. Although there was an increase in level of high density lipoprotein cholesterol (HDL-C) in the test compared to the control group, the increase was nonetheless non-significant (p= 0.068). Our observation of an increase in HDL-C levels is corroborated by other studies on different parts of *C. pepo* that reported similar observation [11,12,21]. HDL-C level has often been positively associated with better cardiovascular health outcome and inversely with atherosclerosis by several clinical studies. Hence, we suggest that the leaves of *C. pepo* may play therapeutic roles in ameliorating or preventing certain cardiovascular diseases.

The preliminary phytochemical screening of the leaves proved it to contain several important phytochemicals of medicinal importance, including flavonoids, saponin, tannin and glycosides. Some of these phytochemicals, isolated mainly from the seeds of *C. pepo*, have been reported to have several medicinal effect, including; antioxidant [22], hepatoprotective [23], antiploriferative [24] and antimicrobial [25] properties.

### 4. CONCLUSION

In conclusion, this work shows that the leaves of *C. pepo* may play an important role in maintaining lipid homeostasis by preserving the optimum balance between the different lipid fractions in the experimental rats here studied. Another interesting outcome of this research effort was the observation of an increase in HDL cholesterol in the rat cohort fed leaves of *C. pepo*. This is exciting considering the importance of high HDL levels in improving cardiovascular health.

Further studies will have to isolate and characterize phytochemical components associated with the observed effect.

### ETHICAL APPROVAL

The animals were handled following standard ethical guidelines on animal handling and research.

### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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