Lipid Profile Normalization Effect of Hunteria Umbellata Aqueous Fruit Extract in Wistar Rats Induced with Aloxan Monohydrate

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ABSTRACT

The present study investigated the potential effect of Hunteria Umbellata fruit extract on lipid profile reduction administered at different daily doses ie 200mg/kg, 400mg/kg compared with control (1) and group 5 treated at 0.3ml standard drug insulin following aloxan monohydrate induction. The lipid profile- HDL, LDL, vLDL, Triglycerides and Total cholesterol were determined using precipitant (mmol), enzymatic hydrolysis, enzymatic and point methods. Results from this study indicate that the lipid profile reduction potential of H.U fruit extract was most effective in group 2 administered 200mg/kg body weight with no aloxan induction. This was closely followed by group 3 treated at 400mg/kg and group 5 treated with the standard drug insulin after aloxan induction compared with the control. However, group 4 induced with aloxan and treated at 200mg/kg body weight has an elevated lipid profile compared with the control and other groups studied. Findings from this study have shown that moderate dose intake of Hunteria umbellata fruit extract by subjects with hyperlipidaemia can be beneficial in the reduction of lipid profile but the rapidity of its reduction rate is dose dependent.

KEYWORDS: Lipid, triglyceride, Hunteria, total cholesterol, high density lipoprotein, hyperlipidaemia.

INTRODUCTION

Lipids are fat-like substances in the body that are essential for proper functioning. They are sources of energy and are stored both in blood and tissues. High level of lipid in the body leads to diseases such as coronary artery disease, stroke or heart attack. While Lipid profile is a panel of blood tests used to assess the level of lipids in the body, it entails four basic parameters thus such as Total cholesterol, Low-density lipoprotein (LDL) cholesterol, High-density lipoprotein (HDL) cholesterol, and Triglycerides.

Total cholesterol

Cholesterol is a sterol type of lipid synthesized by animal cells, it is an essential structural component of the cell membranes. It serves as a precursor for the biosynthesis of steroid hormones, bile acids and vitamin D. It is required to build and maintain membranes and modulates fluidity of the membrane above the range of physiological temperatures.

Through the interaction with the phospholipids fatty-acid chains, cholesterol increase membrane packing, which alters membrane fluidity and maintains membrane integrity so that animal cells do not build cell walls? The membrane remains stable and durable without being rigid, allowing animal cells to change shape and animals to move. (Sadava et al., 2011).

Cholesterol is involve in cell signaling processes that assist in the formation of lipid rafts in the plasma membrane, which brings receptor proteins in close proximity with high concentrations of second messenger molecules (Incardona and Eaton, 2000).

Low-density lipoprotein (LDL) cholesterol

Low-density lipoprotein (LDL) is one of the five major groups of lipoprotein that transport all fat molecules around the body in extracellular water, its particles are formed when triglycerides are removed from VLDL by the lipoprotein lipase enzyme and they become smaller and denser that
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contains higher proportion of cholesterol esters (Pirahanchi et al., 2022) LDL interferes with the quorum sensing system that upregulates genes required for invasive Staphylococcus aureus infection. This mechanism involves the binding of apolipoproteins B to a S. aureus auto inducer pheromone which prevent the signaling through its receptor (Peterson et al., 2008) LDL is grouped based on its size as large low density, its particles are described as pattern A, and small high density LDL particles as pattern B. Pattern B has been associated with a higher risk for coronary heart disease (Ivanova et al., 2017). Because is believed that smaller particles are more easily able to penetrate the endothelium of arterial walls. There has also been noted a correspondence between higher triglyceride levels and higher levels of smaller, denser LDL particles, also lower triglyceride levels and higher levels of the larger, less dense LDL (Superko et al., 2002).

When a cell needs extra cholesterol, it synthesizes the necessary LDL receptors and a proprotein convertase (PCSK9), that marks the LDL receptor for degradation (Zhang et al., 2008), the LDL receptors are inserted into the plasma membrane and diffuse freely to associate with clathrin-coated pits. The LDL receptors bind LDL particles in the bloodstream; the clathrin-coated pits are endocytosed into the cell.

The Vesicles that contain LDL receptors bind to LDL and are delivered to the end some in the presence of low pH. The LDL receptors undergo a conformation change that release LDL, and is shipped to the lysosome, where cholesterol esters in the LDL are hydrolyzed. The LDL receptors are returned to the plasma membrane, where they repeat this cycle. If LDL receptors bind to PCSK9, transport of LDL receptors is redirected to the lysosome, where they are degraded (Santulli et al., 2021).

High-density lipoprotein (HDL) cholesterol

High-density lipoprotein (HDL) is the smallest of the lipoprotein particles and the dense that contains the highest proportion of protein to lipids. Its most abundant apolipoproteins are apo A-I and apo A-II. The liver synthesizes these lipoproteins as complexes of apolipoproteins and phospholipids that resemble cholesterol-free flattened spherical lipoprotein particles (Deng et al., 2022). It’s been documented to be more effective in protecting against and regressing arterial diseases. HDL transports cholesterol to the liver or organs such as adrenals, ovary, and testes by both direct and indirect pathways. Increase concentrations of HDL particles are associated with decrease accumulation of atherosclerosis within the walls of the arteries (Casula et al., 2021), which then reduce the risk of cardiovascular diseases, stroke and other vascular diseases(Deng et al., 2022). HDL particles are said to be good cholesterol because they transport fat molecules out of the arterial walls, reduce macrophage accumulation, and help in the prevention of atherosclerosis.

Studies have shown that very high concentrations of HDL particles can be associated with increased mortality (Madsen et al., 2017), and cardiovascular risk, especially in hypertensive patients (Trimarco et al., 2022).

Triglycerides

These are esters formed from glycerol and three acids. They are tri-esters that consist of a glycerol bound to three fatty acid molecules, and are the main constituents of body fat as well as vegetable fat (Nelson and Cox, 2000), they are present in blood to enable the bidirectional transfer of adipose fat and blood glucose from the liver (Lampe et al., 1983). Triglycerides specific classification focuses on saturated and unsaturated types. Saturated fats have no C=C groups; unsaturated fats feature one or more C=C groups. Unsaturated fats tend to have a lower melting point than saturated analogues; as a result, they are often liquid at room temperature. Triglycerides store unused calories and provide the body with energy. Triglycerides bind to lipoproteins to enable them travel through the blood. It provides the body with energy and store energy for later use. High levels of triglycerides in the blood may lead to atherosclerosis, the clogging and hardening of the arteries. (Gerhard and Eleesha, 2018) It is usually done in fasting blood specimen.

It has been established that Hunteria umbellata in herbal medicine is used for the treatment of diabetes, peptic ulcers, piles, yaws, dysmenorrheal, fevers, infertility, and helminthes infections. (Ighodaro et al., 2013). Hunteria umbellate caused significant increase in the levels of cholesterol, triglycerides, low-density lipoprotein cholesterol, very low-density lipoprotein cholesterol with high reduction in high-density lipoprotein cholesterol level (Ajiboye et al., 2017). It is also established that aqueous seed extract of Hunteria umbellata possesses hypoglycemic, hypolipidemic and antioxidants abilities as evident from its capability to extenuate insulin resistance, dyslipidemia, inflammation and oxidative stress in high-fructose diet-induced metabolic syndrome rats. (Ajiboye et al., 2017).

Studies have shown that Hunteria Umbellata has antihyperlipidaemic effects may partly be mediated through inhibition of intestinal lipid absorption and de novo biosynthesis of cholesterol, thus justifies the ethnopharmacological use of the extract in the management of hyperlipidaemia.

It’s been establish that oral treatments of normal rats with H. umbellata result in significant decreases in serum lipid profile parameters except for high density lipoprotein which increase significantly (triglycerides, total cholesterol and low density lipoprotein. (Adejuwon and Peter, 2015).
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MATERIALS AND METHODS
Fruits and Extraction
The fresh fruits of Hunteria Umbellata were purchased from Ahoada market River State Nigeria. They were weighed and measured in the University of port Harcourt pharmaceutical laboratory (4861g) while still fresh, washed in running tap water, sliced into pieces and immediately macerated in absolute ethanol JHD brand (1.02 kg in 1500 mL absolute ethanol) to quench possible enzymatic action (Harbourne 1973) and left for 72 hours with intermittent shaking. After the period of 72 hours, the ethanol was filtered off and the residue further pulverized using an electric blender and subsequently macerated in 1500 mL absolute ethanol for another 72 hours with intermittent shaking. After filtration, the residual marc was similarly macerated for yet another 72 hours to make room for exhaustive extraction of the metabolites. All the filtrates were combined and concentrated using a rotary evaporator to remove the ethanol and the concentrated extract further dried on a water bath at 50°C and thereafter kept in a desiccator to remove residual moisture to afford the crude ethanol extract used for this study. The yield of the dried crude ethanol extract was then calculated using the formula: % yield = [Weight of dried crude ethanol extract] x 100
[Weight of the fresh fruits of Hunteria Umbellata used]
The extraction and photochemical analysis was done in the Central Laboratory for Phytomedicines, Department of Pharmacognosy and Phytotherapy, Faculty of Pharmaceutical Sciences, University of Port Harcourt by a Principal investigator (Natural products, Analytical and Functional Food Chemistry Research) Nuclei for Phytomedicines and chemical Ecology Research Group

PHYTOCHEMICAL ANALYSIS
The photochemical constituents detected from the fruit extract include alkaloids, phenolic, triterpenoids, carbohydrates and Saponins.

RESEARCH ANIMALS
The Research animals were forty (40) male wistar rats in number weighing between 147 - 312g. The animals were purchased from the animal house of the University of Port Harcourt Faculty of medical Sciences and housed in wire mash cage under environmental condition of 25-29°C. The animals were exposed to 12hours daylight and dark circle according to standard protocols. The research animals were acclimatized for two weeks (14 days) and during the period they were fed with water ad libitum. The standard diet for animal feeds before the induction with aloxan monohydrate. The study was carried out in accordance with Helsinki recommendation in 1983 on guiding principles in care and use of animals.

EXPERIMENTAL DESIGN

Study Design

Table 1. Groupings of the Animals and Dosage Administered

<table>
<thead>
<tr>
<th>Groups</th>
<th>Route of Administration</th>
<th>Daily Dosage Administered (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (control)</td>
<td>Orally-Water and feeds</td>
<td>ad libitum.</td>
</tr>
<tr>
<td>2</td>
<td>Oral</td>
<td>200mg/kg (0.5ml) body weight extract daily and water ad libitum without induction</td>
</tr>
<tr>
<td>3</td>
<td>Orally</td>
<td>400mg/kg (1ml) body weight extract daily after induction</td>
</tr>
<tr>
<td>4</td>
<td>Intraperitoneal and orally</td>
<td>200mg/kg (0.5ml) extract daily and water after single dose intraperitoneal induction with aloxan.</td>
</tr>
<tr>
<td>5</td>
<td>Intraperitoneal</td>
<td>0.3ml insulin</td>
</tr>
</tbody>
</table>

Weight Measurement
The animals weight were measured weekly using an electric scale weight Balance-Golden Meter USA calibrated in grammes during the twenty-eight (28) days period of the study.

Blood Sample Collection
The blood samples were collected directly from the left ventricle of the anaesthetized animals through cardiac
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puncture using a 23G needle mounted on a 5ml syringe plunger. The samples were then introduced into EDTA and plain bottles (15 x 15) purchased from Agari pharmaceuticals LTD China.

**STATISTICAL ANALYSIS**

The statistical analysis was done using statistical packaging for social sciences version 23.0. The results are presented as mean± standard deviation and p-value of <0.05 considered significant.

**Determination of Cholesterol (Enzymatic And-Point Method)**
- Principle: The cholesterol is determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase.
- Procedure: Label the tubes as test, std 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 at 25°C.
- Read and record the absorbance at 540nm. Unit mmol/l

**Determination of Triglycerides (Unit Mmol/L)**
- Principle: The triglycerides are determined after enzymatic hydrolysis with lipases. The indicator is a quinoneimine formed from hydrogen-peroxide, 4 – aminophenazone and 4 – chlorophenol under the catalytic influence of peroxidase.
- Procedure: Label the tubes as test, std and blank.
- Pipette 1.0ml of the rgt into all the tubes
- Add 10ul of std, sample & d/w into appropriate tubes.
- Mix and incubate for 10mins at 25°C.
- Read and record the absorbance at 540nm

**RESULTS**

Table 2. Lipid Profile Of Treated Groups With The Extract And Control

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group1</th>
<th>Group2</th>
<th>Group3</th>
<th>Group4</th>
<th>Group5</th>
<th>Sig (&lt;0.05)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC (mmol/L)</td>
<td>4.45±0.07</td>
<td>3.50±0.56</td>
<td>4.00±0.98</td>
<td>4.65±0.21</td>
<td>4.30±0.14</td>
<td>0.04</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
<td>1.66±0.17</td>
<td>1.59±0.04</td>
<td>1.68±0.05</td>
<td>1.75±0.03</td>
<td>1.40±0.21</td>
<td>0.42</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.49±0.22</td>
<td>1.29±0.05</td>
<td>1.42±0.49</td>
<td>1.62±0.08</td>
<td>1.40±0.03</td>
<td>0.04</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>3.77±0.07</td>
<td>2.94±0.54</td>
<td>3.36±0.95</td>
<td>3.82±0.28</td>
<td>3.54±0.78</td>
<td>0.21</td>
</tr>
<tr>
<td>VLDL (mmol/L)</td>
<td>0.76±0.78</td>
<td>0.73±0.02</td>
<td>0.76±0.02</td>
<td>0.79±0.01</td>
<td>0.64±0.09</td>
<td>0.42</td>
</tr>
</tbody>
</table>

NB: 0.01-0.05 = significant, TC=Total cholesterol, TG=Triglyceride, HDL=High density lipoprotein, LDL=Low density lipoprotein, VLDL=Very low density lipoprotein. Group1 is the control, group2 was treated at 200mg/kg, group3 at 400mg/kg, group4 at 200mg/kg extract daily and group5 was treated with standard drug insulin at 0.3ml daily

Table 3. Correlation Between Total Cholesterol And Other Lipids

<table>
<thead>
<tr>
<th>Measured variables</th>
<th>r-values</th>
<th>p-values</th>
<th>Significant</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC versus TG</td>
<td>0.19</td>
<td>0.65</td>
<td>Non-significant</td>
</tr>
<tr>
<td>TC versus HDL</td>
<td>0.58</td>
<td>0.04</td>
<td>Significant</td>
</tr>
<tr>
<td>TC versus LDL</td>
<td>0.98</td>
<td>0.00</td>
<td>Significant</td>
</tr>
<tr>
<td>TC versus VLDL</td>
<td>0.19</td>
<td>0.59</td>
<td>Non-significant</td>
</tr>
</tbody>
</table>

NB: Significant is at 0.00 to 0.05 (2-tailed).

Table 4. Correlation Between Triglycerides Versus Othr Parameters

<table>
<thead>
<tr>
<th>Measured variables</th>
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</tr>
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<tr>
<td>TG VERSUS TC</td>
<td>0.19</td>
<td>0.58</td>
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<tr>
<td>HDL VS TC</td>
<td>0.65</td>
<td>0.04</td>
<td>Significant</td>
</tr>
<tr>
<td>HDL VS LDL</td>
<td>0.55</td>
<td>0.10</td>
<td>Non-significant</td>
</tr>
<tr>
<td>HDL VS VLDL</td>
<td>0.61</td>
<td>0.06</td>
<td>Non-significant</td>
</tr>
</tbody>
</table>

**DISCUSSION**

This study was carried out to investigate the effect of aqueous fruit extract of Hunteria umbellata on lipid profile using wistar rats induced with aloxan monohydrate. The animals were subjected to two weeks (14 days) acclimatization before induction. Results from the study indicate that all the lipid profiles in group 2 that was not induced with the aloxan monohydrate but were treated at 200mg/kg body weight had lower TC, TG, HDL, LDL, and VLDL compared with control group. However, group 3 treated at 400mg/kg extract following induction had a lower TC, HDL and LDL compared with the control. The lipid profile in group 4 treated at 200mg/kg body weight extract were all higher than the control group while all the lipid profile of group 5 administered the standard drug insulin were significantly lower in comparison with the control.

Results from previous studies have shown the antihyperlipidaemic and cardiovascular protective effect of Hunteria umbellata alkaloids administered at 25 and 50mg/kg daily in hyperlipidaemia induced wistar rats (Adejuwon et al., 2015). Human research studies conducted by Solomon et al., 2017 and 2021 observed significant increase in total cholesterol among adult subjects and pregnant women during the first through third trimesters with resultant increased in blood pressures. Further observation from this study is the positive correlation between total cholesterol, high density lipoprotein and low density lipoprotein with a significant p-value of 0.04 and 0.00 respectively. Other observations from this study indicate that as the triglycerides level increases, the high density lipoprotein and very low density lipoprotein also significantly increases to produce a positive correlation among the lipoproteins. Significant weight reduction in experimental animals induced with aloxan monohydrate and phenylhydrazine treated with Hunteria umbellata fruits extract are now established facts by medical and clinical researchers (Emily et al., 2022; Charles et al., 2022). Clinical studies have shown that decreased serum lipids reverses heart dysfunction while elevated lipid profile increase the risk of non-ischemic heart failure by promoting the development of plaques formation within the intima of blood vessels (Yu Si et al., 2020; Uvoh et al., 2017). Dietary disorders, obesity and abnormal fat metabolism are attributed causes of hyperlipidaemia in patients with cardiovascular diseases. Elevated serum cholesterol reduces coronary blood flow reserved, capillary density which could lead to left ventricular dysfunction.

**CONCLUSION**

The decrease lipoproteins observed in group 3 treated at 400mg/kg extract and group 5 treated with standard drug insulin after with aloxan monohydrate compared with the control (1) group were dose dependent.

**CONFLICT OF INTEREST**

The authors hereby declare that there is no conflict of interest.

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