EVALUATION OF THE ANTI-HYPERLIPIDEMIC ACTIVITY OF THE AQUEOUS ROOT EXTRACT OF ELAEIS GUINEENSIS, JACQ (ARECACEAE)

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ABSTRACT

Elaeis guineensis- Jacq (Areceaceae) is reported for the treatment of a variety of ailments notable amongst these are diabetes and hyperlipidemia. The present study is aimed at evaluating its anti-hyperlipidemic effect, via olive oil induced hyperlipidemia. The aqueous extract was obtained from a decoction of the roots, and later administered to olive oil loaded rats (5 ml/kg). Administration of 250 and 500 mg/kg of the extract was done 30 min before olive oil administration. Distilled water was used as control, while atorvastatin (50 mg/kg) was the standard drug. All administrations were done orally. Blood samples were withdrawn via the abdominal aorta 2 and 4 h after olive oil administration and centrifuged at 3000 rpm for 15 to 20 min. The plasma samples obtained were subjected to biochemical analysis for HDL, LDL, triglycerides and total cholesterol. Acute treatment with the extract points to a significant (p<0.05) reduction in total cholesterol by (35.86 and 56.32%), LDL (61.27 and 20%) and triglycerides (53.87 and 71.23%) by 250 mg/kg at the 2nd and 4th h respectively. At the 500 mg/kg dose, a significant reduction (p<0.05) was also obtained in TC by (36.88 and 34.84%), TG (69.51 and 51.77%) and LDL (66.19 and 50%) at the 2nd and 4th h respectively. While a significant increase (p<0.0001) at both doses of the extract was noted for the HDL by (295.29 and 6.72% for 250; 309.33 and 43.21% for 500 mg/kg) at 2nd and 4th h respectively. The effect of the extract was noted to be more pronounced in the 2nd h in comparison with the 4th h. Phytochemical screening revealed the presence of alkaloids, tannins, flavonoids, terpenoids, and steroids. The plant possesses hypolipidemic effect, considering the increase and decrease in HDL and LDL respectively and thus a useful remedy for hyperlipidemia.

INTRODUCTION

Hyperlipidemia is an elevation of lipids in the bloodstream. These lipids include cholesterol, cholesterol esters, phospholipids and triglycerides. They are transported in the blood as part of large molecules called lipoproteins. Hyperlipidemia is a general term, it could be either high cholesterol in the blood (hypercholesterolemia), high triglycerides in the blood (hypertriglycerideremia) or it could be both [1].

Since hyperlipidemia is usually an elevated level of total cholesterol (TC), triglycerides (TG) and low density lipoprotein (LDL), and decreased level of high density lipoprotein (HDL), treatment is geared towards a reduction in the elevated LDL, TG and TC, alongside an enhancement in the diminished HDL values [1].

Elaeis guineensis Jacq (Areceaceae) is one of the plants that are central to the lives of traditional societies in West Africa. It has been reported as a traditional folkloric medicine for a variety of ailments, these include: skin infection, rheumatism, diabetes, hyperlipidemia, elephantiasis amongst others. It is also claimed to be used as a liminent for indolent tumor [2].

The plant leaves are also used in some parts of Africa for wound healing, but there are not scientific reports on any wound healing activity of the plant. The antimicrobial activity of the extract was examined using the disk diffusion technique and broth dilution method. The plant, Elaeis guineensis, is said to be used in folklore to treat hyperlipidemia[3], hence this study.

The present study evaluated the acclaimed hypolipidemic effect of Elaeis guineensis using rats after induction of a high cholesterol level, and attempted to provide a scientific basis for its use in folk medicine.

RESULTS

Phytochemical screening of the aqueous root extract of Elaeis guineensis.

The preliminary phytochemical screening revealed the presence of carbohydrate, saponin, tannins, flavonoids, alkaloids, terpenoids and steroids. It showed the absence of anthra quinones.

The effect of the aqueous root extract of Elaeis guineensis on total cholesterol level in olive oil induced hyperlipidemia in rats

The aqueous root extract of Elaeis guineensis at the dose of 250 mg/kg significantly decreased (p<0.001) plasma total cholesterol level by 35.86 and 56.32% after 2 and 4 h respectively when compared with the hyperlipidemic group. At 500 mg/kg, a similar significant decrease (p<0.001) in the plasma total cholesterol level was also observed; 36.88 and 34.84% after 2 and 4 h, respectively, in comparison with the hyperlipidemic group. The standard drug, Atorvastatin significantly decreased (p<0.01 and p<0.05) plasma.
total cholesterol level by 34.84 and 46.37% after 2 and 4 h, respectively. It thus appears that the effect of the extract was better than that of atorvastatin. However the reduction of the plasma total cholesterol level by the extract was noted not to be dose dependent, and possesses comparable hypocholesterolemic effect with Atorvastatin. This can be seen in Table 1.

The Effect of the aqueous root extract of *Elaeis guineensis* on Triglyceride level (mg/dl) in olive oil induced hyperlipidemia in rats

Table 2 shows the result of the effect of the aqueous root extract of *Elaeis guineensis*. A significant reduction at the dose of 250 mg/kg (p<0.05, p<0.0001) in plasma Triglyceride level by 53.87 and 71.23% was noted after 2 and 4 h, respectively, when compared with the hyperlipidemic group. A reduction of in TG by 69.51 and 51.77% after 2 and 4 h, respectively, in comparison with the hyperlipidemic group was obtained. A better significance (p<0.05, p<0.0001) was observed with the 500 mg/kg dose at the 2nd h, though the effect of 500 mg/kg at the 4th h, was not better than that of the 250 mg/kg, again pointing to a non-dose dependent effect. However the effect of atorvastatin was not significant after the 2nd h though a reduction was observed. Its effect after 4 h, was significant (p<0.0001), as a decrease in plasma TG level by 59.76% was observed. The extract thus appears to have a better hypotriglyceridemic effect.

Effect of aqueous root extract of *Elaeis guineensis* on high density lipoprotein (HDL) the olive oil induced hyperlipidemia

The aqueous root extract of *Elaeis guineensis* at the dose of 250 mg/kg significantly increased (p<0.01) plasma HDL by 295.29% after 2 h, and insignificantly increased plasma HDL by 6.72% after 4 h, when both compared with hyperlipidemic group (Table 3). The extract at the dose of 500 mg/kg significantly increased (p<0.01) plasma HDL by 309.33 and 43.21% (p<0.05) after 2 and 4 h respectively, when compared with hyperlipidemic group. The effect of the extract was noted to be most prominent after 2 h. Atorvastatin (50 mg/kg) insignificantly increased plasma HDL level by 21.61% after 2 h and significantly increased (p<0.01) it by 117.5% after 4 h.

Effect of aqueous root extract of *Elaeis guineensis* on plasma low density lipoprotein (LDL) level in hyperlipidemic rats.

The results are presented in Table 4. The aqueous root extract of *Elaeis guineensis* at the dose of 250 mg/kg significantly decreased (p<0.01) plasma LDL level by 61.27 and 20.0% after 2 and 4 h respectively when compared with hyperlipidemic group, while at 500 mg/kg a similar significant decrease (p<0.01) 66.19 and 50.0% after 2 and 4 h respectively in comparison with hyperlipidemic group was observed. The standard drug, Atorvastatin at the dose of 50 mg/kg significantly decreased (p<0.01) plasma LDL level by 67.60 and 87.50% at the 2nd and 4th h, respectively when compared with hyperlipidemic group.

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>TC after 2 hrs (mg/dl)</th>
<th>TC after 4 hrs (mg/dl)</th>
<th>% reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (2 ml/kg)</td>
<td>33.75 ± 3.71</td>
<td>24.00 ± 2.00</td>
<td>-</td>
</tr>
<tr>
<td>OL (5 ml/kg)</td>
<td>73.67 ± 0.33*</td>
<td>43.50 ± 2.50*</td>
<td>-</td>
</tr>
<tr>
<td>OL + E.G (250)</td>
<td>47.25 ± 2.29*</td>
<td>19.00 ± 3.08*</td>
<td>35.86 56.32</td>
</tr>
<tr>
<td>OL + E.G (500)</td>
<td>46.50 ± 3.00*</td>
<td>16.67 ± 3.18*</td>
<td>36.88 61.68</td>
</tr>
<tr>
<td>OL + AV (50)</td>
<td>48.00 ± 3.70*</td>
<td>22.33 ± 3.38*</td>
<td>34.84 46.37</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M for the animals in each group.

n = 10 per group. *p<0.05 significantly higher than control, †p<0.001 and ‡p< 0.01 significantly lower as compared with the hyperlipidemic group.

OL: Olive oil alone (Hyperlipidemic)

E.G: Aqueous extract of *Elaeis guineensis* ; AV: Atorvastatin
Table 2: The Effect of the aqueous root extract of *Elaeis guineensis* on Triglyceride level (mg/dl)

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>TG after 2 hrs (mg/dl)</th>
<th>TG after 4 hrs (mg/dl)</th>
<th>% reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (2 ml/kg)</td>
<td>15.00 ± 4.00</td>
<td>42.50 ± 10.50</td>
<td>-</td>
</tr>
<tr>
<td>OL (5 ml/kg)</td>
<td>62.33 ± 8.11**</td>
<td>104.3 ± 4.17**</td>
<td>-</td>
</tr>
<tr>
<td>OL + E.G (250)</td>
<td>28.75 ± 2.86c</td>
<td>30.33 ± 2.84c</td>
<td>53.87</td>
</tr>
<tr>
<td>OL + E.G (500)</td>
<td>19.00 ± 2.61b</td>
<td>50.33 ± 5.84c</td>
<td>69.51</td>
</tr>
<tr>
<td>OL + AV (50)</td>
<td>51.33 ± 9.28</td>
<td>42.00 ± 2.00</td>
<td>17.64</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M for the animals in each group.

n = 10 per group. **p<0.001 significantly higher than control, ‘p<0.0001 and ‘p<0.01 significantly lower as compared with the hyperlipidemic group, and ”p< 0.01 significantly different from the atorvastatin group

OL: Olive oil alone (Hyperlipidemic); E.G: Aqueous extract of *Elaeis guineensis*; AV: Atorvastatin

Table 3: The Effect of the aqueous root extract of *Elaeis guineensis* on HDL level (mg/dl)

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>HDL after 2 hrs (mg/dl)</th>
<th>HDL after 4 hrs (mg/dl)</th>
<th>% increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (2 ml/kg)</td>
<td>23.25 ± 1.38</td>
<td>27.33 ± 0.33</td>
<td>-</td>
</tr>
<tr>
<td>OL (5 ml/kg)</td>
<td>14.25 ± 2.46a</td>
<td>24.67 ± 1.86</td>
<td>-</td>
</tr>
<tr>
<td>OL + E.G (250)</td>
<td>56.33 ± 7.36b</td>
<td>26.33 ± 1.45</td>
<td>295.29</td>
</tr>
<tr>
<td>OL + E.G (500)</td>
<td>58.33 ± 11.60c</td>
<td>35.33 ± 1.15</td>
<td>309.33</td>
</tr>
<tr>
<td>OL + AV (50)</td>
<td>17.33 ± 2.90</td>
<td>53.67 ± 14.84b</td>
<td>21.61</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M for the animals in each group.

n = 10 per group. *p<0.05 significantly lower than control, ‘p<0.01 significantly higher as compared with the hyperlipidemic group, and ”p< 0.01 significantly different from the atorvastatin group

OL: Olive oil alone (Hyperlipidemic); E.G: Aqueous extract of *Elaeis guineensis*; AV: Atorvastatin

Table 4: The Effect of the aqueous root extract of *Elaeis guineensis* on LDL level (mg/dl)

<table>
<thead>
<tr>
<th>Treatment (mg/kg)</th>
<th>LDL after 2 hrs (mg/dl)</th>
<th>LDL after 4 hrs (mg/dl)</th>
<th>% reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (2 ml/kg)</td>
<td>0.50 ± 0.10</td>
<td>3.80 ± 0.50</td>
<td>-</td>
</tr>
<tr>
<td>OL (5 ml/kg)</td>
<td>7.10 ± 0.11**</td>
<td>4.00 ± 0.17</td>
<td>-</td>
</tr>
<tr>
<td>OL + E.G (250)</td>
<td>2.75 ± 0.86b</td>
<td>3.20 ± 0.84</td>
<td>61.27</td>
</tr>
<tr>
<td>OL + E.G (500)</td>
<td>2.40 ± 0.61b</td>
<td>2.00 ± 0.84</td>
<td>66.19</td>
</tr>
<tr>
<td>OL + AV (50)</td>
<td>2.30 ± 0.28b</td>
<td>0.50 ± 0.01c</td>
<td>67.60</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M for the animals in each group.

n = 10 per group. **p<0.001 significantly higher than control, ‘p<0.001 and ‘p< 0.01 significantly lower as compared with the hyperlipidemic group.

OL: Olive oil alone (Hyperlipidemic); E.G: Aqueous extract of *Elaeis guineensis*; AV: Atorvastatin
DISCUSSION

Hyperlipidemia is an elevation of lipids in the bloodstream. These lipids include cholesterol, cholesterol esters, phospholipids and triglycerides. They are transported in large molecules called lipoproteins [1].

In humans, cholesterol is the metabolic precursor of steroid hormones (cortisol, aldosterone, estrogens and androgens) that regulate a great variety of physiologic functions. High cholesterol level (above 200 mg/dl) is a risk factor for coronary heart disease. It causes the build-up of plaque in the artery and this may result in narrowing of the vessel leading to high blood pressure or complete blockage of the vessel which result in heart attack. As the level of cholesterol rises above 180 mg/dl (optimum level), the risk for developing coronary heart disease increases and a reduction of 1% is shown to reduce the risk for coronary heart disease by 2% for level over 200 mg/dl. Hypercholesterolemia may be related to diet, genetic factors (such as LDL receptor mutations in familial hypercholesterolemia) and the presence of other diseases such as diabetes and under active thyroid [4].

Triglycerides are esters derived from glycerol and three fatty acids which could be saturated or unsaturated [5]. In the human body, high levels of triglycerides (above 150 mg/dl.) in the bloodstream have been linked to atherosclerosis and by extension, the risk of heart diseases and stroke.

Low density lipoprotein (LDL) is also known as bad cholesterol which clogs the arteries when oxidised. The recommended maximum level of low density lipoprotein cholesterol is 129 mg/dl where less than 100 mg/dl is considered optimal and anything above 160 mg/dl is considered high and a risk factor for cardiovascular diseases.

High density lipoprotein (good cholesterol) is high density lipids that help keep the blood vessels clear of plaques. It actually attracts the low density lipoprotein, binds with it and flushes it out of the body through the intestinal tract. Healthy adults need a minimum of 40 mg/dl for men and 50 mg/dl for women. Low level of high density lipoprotein below the minimum value is a risk factor for heart diseases.

The aqueous root extract of *Elaeis guineensis* revealed a significant reduction in plasma cholesterol, triglycerides, low density lipoprotein levels and resulted in an increase in high density lipoprotein.

It has also been shown that there is relationship between elevated LDL and atherosclerosis, since LDL in the blood gets deposited in the blood vessel walls and become a major component of atherosclerotic plaque. Studies suggest that pathological process could be reversed by reducing the serum LDL level [10]. Since the aqueous root extract of *Elaeis guineensis* significantly reduced cholesterol and LDL levels, therefore, it could be said that the aqueous root extract of *Elaeis guineensis* might prevent atherosclerosis. Moreover acute treatment with the aqueous root extract brought about significant increase in plasma HDL levels which is considered to be good cholesterol. The increase in HDL level has been shown to slow down atherosclerosis process [11]. It could be therefore said that the aqueous root of *Elaeis guineensis* has a protective action in hyperlipidemic rats. This protective action may result from the participation of HDL in reverse cholesterol transport. These function include putative anti-inflammatory, anti-oxidative, platelet anti-aggregation, anti-coagulant and fibrolytic activities [11].

It has been investigated that Olive oil induced hyperlipidemia (hypertriglyceridemia) in rats is by the secretion of chylomicron-triglyceride complexes from the intestine into the lymph, thereby increasing plasma triglyceride level [1]. Since the results of this study shows that the aqueous root extract of *Elaeis guineensis* significantly decreased plasma triglyceride levels in rats after olive oil administration, the possible mechanism of reduction of triglycerides by the extract may be by suppressing the secretion of chylomicron-triglyceride complexes into the lymph.

Olive oil may also induce hyperlipidemia (hypercholesterolemia) by the inhibition of lecithin cholesterol acyl transferase (LCAT) which play a key role in the incorporation of free cholesterol into the HDL and transferring it back to very low density lipoprotein (VLDL) and low density lipoprotein (LDL) which are taken back later in liver cell [12]. Therefore another possible mechanism of lipid lowering activity of the extract may be due to the enhancement of the activity of lecithin acyl transferase and the inhibition of hepatic triglyceride-lipase on HDL [13].

MATERIALS AND METHODS

Plant material

The root of *Elaeis guineensis* was collected from the University of Benin, Benin City, Edo State Nigeria in July 2012. Identification of the plant was done by Mr. Sunny Nweke a herbarium curator in the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria where a herbarium specimen exists. Immediately after collection, the roots were air-dried and then pulverised with the aid of an impact mill. The powdered sample was weighed and kept for further analysis.

Drug and Chemicals

Thiopentone (Samarth Life Sciences), Atorvastatin (Pfizer PLC), olive oil (Goya en espana, S.A.U Sevilla, Spain), Distilled water
Animals
Swiss albino rats weighing 150-280 g of either sex kept at the Laboratory Animal house of the Department of Anatomy, University of Benin were used. The animals maintained under standard environmental conditions had access to standard diet (Top feed) and water ad libitum. Animals were kept in a cage with a 12 h light/dark cycle. The animals were fasted 12-14 h before experimentation but were allowed free access to water.

Ethical approval
Approval for the use of the animals was obtained from the ethical committee on the use of animals, Faculty of Pharmacy, University of Benin, Benin City, Edo State, Nigeria. Ethical approval letter dated November 22nd, 2012.

Extraction of Plant Material
The powdered plant (400 g) was mixed with 5 L of water, boiled for 30 min on a hot plate. The resulting mixture was allowed to cool and then filtered. The resulting solution was re-filtered using cotton wool plugs in a funnel to obtain a clear solution which was then concentrated to dryness over to obtain a brown coloured residue. The residue was weighed (15.5 g) and the yield calculated as (3.9%).

Phytochemical screening
Qualitative chemical test were performed to assess the presence of various phytochemical constituents of the aqueous root extract of *Elaeis guineensis* [14].

Screening for anti-hyperlipidemic activity
Fasted rats were divided into five groups of 10 rats each. Group 1 served as the control and received distilled water at the dose of 2 ml/kg. Group 2 was kept as hyperlipidemic and administered with olive oil only. Group 3 and 4 were treated with the aqueous extract at the oral dose of 250 and 500 mg/kg respectively. Animals in group 5 received atorvastatin (50 mg/kg). Olive oil (5 ml/kg oral dose) was administered 30 min after drug/extract treatment [15, 1]. All administrations were done orally.

The first five animals in each group were anaesthetized with thiopentone (40 mg/kg) and then sacrificed after which blood samples were collected via the abdominal aorta, 2 h after olive oil treatment. While the last five rats were sacrificed 4 h after olive oil treatment and blood samples also withdrawn via the abdominal aorta following anaesthesia with thiopentone.

The blood samples were transferred immediately into lithium heparin tubes and then centrifuged at 4000 rpm for 5 to 10 min. Clear plasma thus obtained was transferred carefully with the aid of micropipette into small test tube for estimation. The plasma concentration of total cholesterol, HDL and triglyceride were measured by standard procedures using colorimetric methods [16].

Total cholesterol
Total cholesterol in plasma was determined by enzymatic method, using wet reagent diagnostic kits, which is a modification of the method of Abell [17, 18, 19]. The reagent (1000 µl) was pipetted into 3 different test tubes A, B and C. Distilled water, a standard solution (standard) and plasma (sample) of 100 µl each were pipetted into same test tubes A, B and C respectively. After mixing, the test tubes were left to stand for 10 minutes at room temperature to allow for colour change, after which the absorbance of the blank, standard and samples were read at 500 nm using a colorimeter. This was repeated for all the plasma samples. Concentration of cholesterol in sample was then calculated and expressed in mg/dl.

Triglycerides
Total triglyceride in plasma was determined by the enzymatic method, using wet reagent diagnostic kits, a modification of the method of Jacobs and Vandemark [20, 21]. The reagent (1000 µl) was pipetted into 3 different test tubes A, B and C Distilled water, a standard solution (standard) and plasma (sample) of 10 µl each were pipetted into same test tubes A, B and C respectively. After mixing, the test tubes were left to stand for 10 minutes at room temperature to allow for colour change. The absorbance of the blank, standard and sample were read 500 nm using a colorimeter. This was repeated for all the plasma samples. Concentration of the triglyceride in sample was then calculated and expressed in mg/dl.

High density lipoprotein
Accurate measurement was done by the enzymatic method, using wet reagent diagnostic kits. A precipitating reagent was used. This was pre-diluted in the ratio 4 to 1 with distilled water. This reagent composition is phosphotungstic acid: (0.55mmol/l) and magnesium chloride: (25 mmol/l) [22].

The reagent (500 µl) was pipetted into 2 different test tubes A and B. A standard solution (standard) and plasma (sample) of 200 µl each were pipetted into same test tubes A and B respectively. After mixing, the test tubes were left to stand for 10 min at room temperature to allow for colour change, after which the absorbance of the standard and sample were read at 500 nm using a colorimeter. This was repeated for all the plasma samples. Concentration of HDL in sample was then calculated and expressed in mg/dl.

Low density lipoprotein
This was obtained from the values of total cholesterol, HDL and triglycerides for each of the sample using the formula below [12]In mg/dl:

\[
LDL = \frac{Total\ cholesterol - Triglycerides}{HDL}\times 5
\]

Statistical analysis
Statistical evaluation of the data was done by Student’s ‘t’ test (Graph PAD instat software, version 2.05a). A value from P<0.05 was considered to be significant.
CONCLUSION

The acute treatment with aqueous root extract of *E. guineensis* produced a significant reduction both on TC and TG after olive oil administration. The maximum inhibitory effect on TG and TC level was observed with 500 mg/kg of the aqueous extract of *Elaeis guineensis*. The plant showed protective action as it significantly increased the HDL cholesterol level and decreased LDL level.

The present investigation may be quite useful as this plant is highly valued in traditional system of medicine and can be used to control the state of hyperlipidemia.

Declaration of interest
The authors report no declarations of interest.

REFERENCES