INTRODUCTION
Cardiovascular diseases are constituting a growing health problem all over the world (1). Several factors such as high caloric diet intake, age, lack of exercise, smoking, alcohol consumption and genetic predisposition have been linked with these diseases (2, 3).
Hyperlipidemia is known to be a risk factor for cardiovascular diseases, one of the leading causes of mortality and morbidity in human (4, 5). The modification of lipid concentration has been found to be a useful approach to decrease cardiovascular mortality through prevention of development of atherosclerotic diseases (5).
Many orthodox drugs - niacin, fibrates and 5- hydroxy-3-methylglutaryl-CoA
reductase inhibitors (HMGC\(\text{CoA}\) reductase) among others, are currently in use in the treatment of hyperlipidemia. However, these synthetic medications are not without their adverse effects. As a result of this, the quest for herbal medicines which have lipid-lowering potentials and minimal or no side effects is on the increase.

Medicinal plants have continued to play important roles in the effective delivery of health care in many developed and less developed countries alike. It has been estimated that about 25% of all prescribed medicines today are substances derived from plants (6) and about 80% of the world inhabitants rely mainly on traditional medicines for their primary health care (7).

*Persea americana* pulp is rich in monounsaturated fat, vitamin E, lutein, zeaxanthin, alpha-carotene, beta-carotene (8); tannins (9); the leaf contains flavonoids (10); seed has low protein (11), fibre, vitamins C and K (12); vitamins A, B, potassium, calcium, iron, magnesium, copper, fluorine, lecithin, beta-sitosterol, glucothione, carbohydrate, tannins and oil (which is expressed from the flesh) are also contained in the plant (9). The leaves are astringent and antidiysenteric and are used in the treatment of stomach ulcer (13), hypertension (14), as analgesic and anti-inflammatory (15); anticonvulsant (16); hypoglycaemic and hypocholesterolaemic (17); and vasorelaxant and blood pressure reducing agent (14, 18) in animal studies.

The seed has been reported to have blood pressure-lowering effect (19). A decoction of the stem bark is taken to relieve cough, as an aphrodisiac, emmenagogue, parasitic skin disease agent (14) and in induction of diuresis (20).

The edible fruit pulp is used in wound healing, as an aphrodisiac and as emmenagogue (13), and also has hepatoprotective activity (21).

While studies on the antihyperlipidemic properties of the seed of the plant have focused on the crude extracts (3, 22), we are not aware of any scientific report on the use of the ethyl acetate fraction on the treatment of hyperlipidemia. This present study was therefore designed to evaluate the effects of the ethyl acetate fraction of methanolic seed extract of *Persea americana* on plasma lipids of hyperlipidemic rats as a possible treatment for hyperlipidemia.

**METHODOLOGY**

**Plant material and extraction**

The matured fruits of *Persea americana* were collected from Ifite-Oraifite in Ekwusigo L.G.A of Anambra State in the month of June, 2011. Although a common plant, it was authenticated by the Forest Research Institute of Nigeria, Ibadan where the herbarium sample with voucher number FHI 108336 has been deposited. The seeds were removed from the fruits, chopped and sun-dried for 5 days. The dried seeds were then powdered using a laboratory mill (Christy and Norris Ltd, England).

The powder (645 g) was macerated in 3.225 litres of methanol for 24 hours. It was then, decanted and filtered to remove residues. Filtrate was concentrated by drying in an oven at 30°C for 3 days. A semi-solid paste was obtained by this process (yield=12%w/w). The dried extract (dark-brown in colour) was
preserved in a bottle and stored in a refrigerator at 4°C.

**Animals**
Male Sprague-Dawley rats (170-210 g) of either sex were obtained from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

They were housed in standard cages and fed rat chow (Bendel Feeds and Flour Mill Nigeria Ltd., Ewu), and drinking fluid *ad libitum*. The animals were exposed to natural lighting condition, room temperature, and were handled according to international protocols (23). In this study, animals were fasted for 12-14 h and assigned to five groups with five (5) animals in each group. Induction of hyperlipidemia was done by the oral administration of 5 ml/kg of olive oil to each animal (24). Group 1 served as negative control which received only distilled water (2 ml/kg), while group 2 served as the hyperlipidemic animals which received olive oil (5 mg/kg) and distilled water. Group 3 served as positive control which was treated with atorvastatin (50 mg/kg). Groups 4 and 5 were treated with different doses (250 and 500 mg/kg rat body weight respectively) of ethyl acetate fraction of methanolic extract of the seeds of *Persea americana*. The extract fraction, distilled water, atorvastatin and olive oil were administered to the rats by gastric intubation. All treatments were given 30 min before the administration of olive oil in all the groups (24).

**Collection of blood and plasma and determination of plasma lipids and blood glucose**

Total plasma cholesterol, triglycerides, high density lipoproteins, and glucose were estimated in all groups of animals by collecting about 5 ml of blood through abdominal aortae under thiopentone (40 mg/kg) anaesthesia, 2 h after olive oil treatment.

The whole procedure as described above was repeated and blood sample collected, 4 h after olive oil treatment.

Blood samples were introduced into lithium heparin and fluoride oxalate tubes for analysis of lipids and glucose respectively. The samples were then centrifuged at 4000 revolutions per minute for 10 minutes to obtain the plasma. The samples were analyzed by measuring absorbance using UV spectrophotometer (Spectrum Lab 22 CP), and using diagnostic kits (Randox Laboratories Ltd, United Kingdom) according to manufacturer’s protocol. Total cholesterol (TC), triglyceride (TG), high density lipoprotein (HDL-C), low density lipoprotein (LDL-C), arterogenic index of plasma (AIP) and blood glucose were determined according to standard methodologies (25–32).

**Fractionation of methanolic extract**
The crude extract was diluted by addition of 150 ml distilled water to obtain aqueous solution. The aqueous solution was three times treated with 50 ml ethyl acetate. The upper fraction, representing ethyl acetate fraction, was collected each time using separating funnel. The fractions were combined, air-dried and stored at 4°C in the refrigerator for the experiments (23).

**Statistical analysis**
Data are presented as mean ± SEM (standard error of the mean) and *n*
represents the number of animals used for a particular experiment. Comparisons were made between treated and control groups by use of Student’s t-test. All data were analyzed using GraphPad Instat software (USA). $P < 0.05$ indicates statistically significant difference.

RESULTS AND DISCUSSION

The results of this study are shown on Tables 1-6.

Effect of extract on TC

Total cholesterol was increased by olive oil after both 2 and 4 hours. After 2 h of administration, atorvastatin reduced the level of TC induced by the olive oil. The degree of reduction further increased after 4 h. The reductions were however, not significant (Table 1).

Effect of extract on TG

Table 2 shows the effect of the ethyl acetate fraction on TG. Olive oil increased the level of TG after both 2 and 4 hours. TG was significantly ($p < 0.05$, $P < 0.01$) increased by olive oil at the 2nd and 4th hours respectively, when compared to distilled water group. The increase in TG by the olive oil was more pronounced at the 4th hour. Atorvastatin decreased the level of TG after 2 and 4 hours. EAF (250 mg/kg) significantly ($p < 0.05$) reduced the triglycerides from 86.069 ± 17.361 mg/dl to 39.137 ± 9.287 mg/dl (54.53 %) at the 2nd h and also significantly ($p < 0.01$) from 141.975 ± 3.856 mg/dl to 82.098 ± 8.924 mg/dl (42.17 %) at the 4th h. The extract reduced the triglycerides more than atorvastatin; 64.323 ± 11.676 mg/dl at 2nd h (25.27 %) and 88.770 ± 3.856 mg/dl at 4th h (37.47 %). Increase in the dose of the extract did not enhance the effect; the hypolipidemic activity appeared to have been lost at 500 mg/kg dose.

Effect of extract on HDL

HDL was decreased by olive oil when compared to the distilled water group at the 2nd hour. There was very little increase in HDL level after 4 hours. There was increase in HDL both at the 2nd and 4th hours by atorvastatin. The extract at 250 mg/kg increased HDL at the 4th h, although not significantly (Table 3).

Effect of extract on LDL

Table 4 shows the effect of the ethyl acetate fraction of the extract on LDL. The extract significantly reduced the LDL both at 250 mg/kg ($p < 0.01$) at the 4th hour and at 500 mg/kg ($p < 0.04$ and $p < 0.02$ at the 2nd and the 4th hours respectively). Atorvastatin also significantly ($p < 0.03$) reduced the LDL after 2 hours.

Effect of extract on AIP

The distilled water group (2 hours group) had a very low cardiovascular risk ($AIP < -0.03$). The cardiovascular risk of the distilled water group is significantly ($P < 0.05$) different when compared to the olive oil group. Atorvastatin (2 hours group) had an intermediate ($0.1 < AIP < 0.24$) cardiovascular risk but a lower risk when compared to the olive oil group. There is high cardiovascular risk ($AIP > 0.24$) in olive oil group both at the 2nd and 4th hours. There is intermediate cardiovascular risk ($0.1 < AIP < 0.24$) with EAF (250 mg/kg) at the 4th hour. The AIP was significantly ($p < 0.01$) reduced by the extract (250 mg/kg) at the 2nd h from 0.319 ± 0.097 mg/dl to -0.435 ± 0.692 mg/dl (Table 5). At high dose of the fraction, there was no better effect.
Table 1: Effect of ethyl acetate fraction of *Persea americana* methanolic seed extract on the TC level (mg/dl) of rats.

<table>
<thead>
<tr>
<th>Groups (mg/kg)</th>
<th>TC after 2 hours</th>
<th>TC after 4 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW (2 mg/ml)</td>
<td>49.561±4.959</td>
<td>45.979±3.156</td>
</tr>
<tr>
<td>Olive oil (5 mg/ml)</td>
<td>65.801±14.926</td>
<td>47.793±2.474</td>
</tr>
<tr>
<td>ATV (50)</td>
<td>46.158±11.525</td>
<td>42.489±3.788</td>
</tr>
<tr>
<td>EAF (250)</td>
<td>57.612±7.223</td>
<td>52.021±6.427</td>
</tr>
<tr>
<td>EAF (500)</td>
<td>58.909±10.412</td>
<td>50.587±3.018</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, ATV = Atorvastatin, EAF = Ethyl acetate fraction of *Persea americana* seed and DW = Distilled water, n = 5 per group.

Table 2: Effect of ethyl acetate fraction of *Persea americana* methanolic seed extract on the TG level (mg/kg) of rats.

<table>
<thead>
<tr>
<th>Groups (mg/kg)</th>
<th>TG after 2 hours</th>
<th>TG after 4 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW (2 mg/ml)</td>
<td>33.333±3.734*</td>
<td>68.861±10.597**</td>
</tr>
<tr>
<td>Olive (5 mg/ml)</td>
<td>86.069±17.361</td>
<td>141.975±11.934</td>
</tr>
<tr>
<td>ATV (50)</td>
<td>64.323±11.676</td>
<td>88.770±3.856**</td>
</tr>
<tr>
<td>EAF (250)</td>
<td>39.137±9.287*</td>
<td>82.098±8.924**</td>
</tr>
<tr>
<td>EAF (500)</td>
<td>144.031±15.739*</td>
<td>137.173±13.970**</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM, * p<0.05, ** p<0.01, significantly different when compared to olive oil/water group. ATV = Atorvastatin, EAF = Ethyl acetate fraction of *Persea americana* seed and DW = Distilled water. n = 5 per group.
Table 3: Effect of ethyl acetate fraction of *Persea americana* methanolic seed extract on the HDL level of rats.

<table>
<thead>
<tr>
<th>Group(mg/kg)</th>
<th>HDL after 2 hours</th>
<th>HDL after 4 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW(2 mg/ml)</td>
<td>41.105±3.596</td>
<td>35.277±7.414</td>
</tr>
<tr>
<td>Olive (5 mg/ml)</td>
<td>35.354±2.917</td>
<td>36.111±2.041</td>
</tr>
<tr>
<td>ATV 50</td>
<td>41.614±6.996</td>
<td>50.691±7.450*</td>
</tr>
<tr>
<td>EAF 250</td>
<td>32.331±6.188</td>
<td>42.225±2.842</td>
</tr>
<tr>
<td>EAF 500</td>
<td>32.866±3.571</td>
<td>33±3.151</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SEM, * P<0.05 significantly different when compared to olive oil/water group, ATV =Atorvastatin, EAF=Ethyl acetate fraction of *Persia americana* seed and DW=Distilled water. n = 5 per group

Table 4: Effect of ethyl acetate fraction of *Persea americana* methanolic seed extract on the LDL level of rats.

<table>
<thead>
<tr>
<th>Groups(mg/kg)</th>
<th>LDL after 2 hours</th>
<th>LDL after 4 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW(mg/ml)</td>
<td>0.754±8.365</td>
<td>5.926±6.747</td>
</tr>
<tr>
<td>Olive/water(mg/ml)</td>
<td>30.659±19.066</td>
<td>28.286±9.486</td>
</tr>
<tr>
<td>ATV 50</td>
<td>-21.300±6.009**</td>
<td>13.489±7.764</td>
</tr>
<tr>
<td>EAF 250</td>
<td>22.668±7.080</td>
<td>-11.812±1.654****</td>
</tr>
<tr>
<td>EAF 500</td>
<td>-8.906±3.817*</td>
<td>-9.087±2.780***</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SEM, *p< 0.04, ** p< 0.03, *** < 0.02, ****p< 0.01 significantly different when compared to olive oil/water group, ATV =Atorvastatin, EAF=Ethyl acetate fraction of *Persia americana* seed and DW=Distilled water. n = 5 per group
Table 5: Effect of ethyl acetate fraction of *Persea americana* methanolic seed extract on the AIP of rats.

<table>
<thead>
<tr>
<th>Groups (mg/kg)</th>
<th>AIP after 2 hours</th>
<th>AIP after 4 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW (2 mg/ml)</td>
<td>-0.071±0.077*</td>
<td>0.304±0.233</td>
</tr>
<tr>
<td>Olive (5 mg/ml)</td>
<td>0.319±0.097</td>
<td>0.363±0.157</td>
</tr>
<tr>
<td>ATV (50)</td>
<td>0.235±0.131</td>
<td>0.419±0.093</td>
</tr>
<tr>
<td>EAF (250)</td>
<td>-0.435±0.692*</td>
<td>0.221±0.032</td>
</tr>
<tr>
<td>EAF (500)</td>
<td>0.558±0.094</td>
<td>0.387±0.132</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SEM, * P<0.05 significantly different when compared to olive oil group, ATV = Atorvastatin, EAF = Ethyl acetate fraction of *Persia americana* seed and DW = Distilled water. n = 5 per group.

Table 6: Effect of ethyl acetate fraction of *Persea Americana* methanolic seed extract on the glucose (mg/dl) level of rats.

<table>
<thead>
<tr>
<th>Group (mg/kg)</th>
<th>Glucose level after 2 h</th>
<th>Glucose level after 4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>DW (2 mg/ml)</td>
<td>96.035±14.215</td>
<td>67.592±4.485 **</td>
</tr>
<tr>
<td>Olive (5 mg/ml)</td>
<td>109.46±7.307</td>
<td>90.843±3.671</td>
</tr>
<tr>
<td>ATV 50</td>
<td>76.506±7.628*</td>
<td>67.005±4.657*</td>
</tr>
<tr>
<td>EAF 250</td>
<td>84.248±12.302</td>
<td>80.940±5.445</td>
</tr>
<tr>
<td>EAF 500</td>
<td>98.140±11.916</td>
<td>55.015±3.098 ***</td>
</tr>
</tbody>
</table>

Values are expressed as mean ±SEM, * P<0.05, ** P<0.001, *** P<0.0001 significantly reduced when compared to olive oil group, ATV = Atorvastatin, EAF = Ethyl acetate fraction of *Persia americana* seed and DW = Distilled water. n = 5 per group.
Effect of extract on glucose
Plasma glucose was also significantly (p < 0.0001) reduced by ethyl acetate fraction (250 mg/kg) at the 4th h from 90.843 ± 3.671 mg/dl to 55.015 ± 3.098 mg/dl. This was more than the effect of atorvastatin (Table 6).

Our present study shows that hyperlipidemia was induced by olive oil. It has been shown that olive oil, induced hyperlipidemia in rats by the secretion of chylomicron-triglycerides complexes into the lymph (24). It may induce hyperlipidemia by inhibition of lecithin cholesterol acyl transferase (LCAT) (34). The result shows that level of serum total cholesterol was increased by olive oil. The increase in the total cholesterol, TG and the LDL levels of the olive oil-treated rats observed in this study are in accordance with an earlier report documenting increased plasma TG, LDL and cholesterol level in diabetic subject (35). The total cholesterol and LDL reducing potential of avocado has been attributed to high content of monounsaturated fatty acids, betasitosterol, carotenoids (e.g. zeaxanthin, alphacarotene, beta carotene and tocopherols (Vit. E) present in the pulp of this fruit (36). The original studies of Keys et al. suggested that monounsaturated fats have neutral influence on blood cholesterol concentrations leading to neither a rise nor a fall when administered to volunteers. High levels of monounsaturated and polyunsaturated fatty acids could be responsible for the cholesterol lowering effect of avocado seeds (37).

From the result, it is clear that TG was increased by olive oil. The triglyceride level was reduced significantly (P<0.01) by the standard drug (Atorvastatin).

Atorvastatin is a HMG-CoA reductase inhibitor. HMG-CoA reduces serum triglyceride levels through the modulation of apolipoprotein C-III and lipoprotein lipase (38). The significant reduction in TG of the rats when treated with ethyl acetate fractions of seeds of *Persea americana* in this study supports the findings that avocado consumption decreases serum triglyceride levels in rats (39). The plant constituents like steroids and flavonoids, are reported to possess lipid-lowering activity. The plant steroids reduce the absorption of cholesterol and thus increase fecal excretion of cholesterol. Flavonoids augument the activity of lecithin acyltransferase (LCAT) which regulates blood lipids (40).

HDL was increased by both atorvastatin and *Persea americana* fractions. Olive oil reduced the level of HDL. Ethyl acetate fractions also increased the level of HDL which may probably be due to presence of flavonoids (41). Flavonoids are reported to increase HDL-concentration and decrease LDL and VLDL levels in hyperlipidemic rats (42). Increases in plasma HDL cholesterol have been considered to reduce risk in coronary heart disease (43). High HDL exerts a protective effect by decreasing the rate of entry of cholesterol into the cell via LDL i.e. by enhancing reverse cholesterol transport by scavenging excess cholesterol from peripheral tissues followed by esterification through lecithin: cholesterol acyltransferase and delivering it to the liver and steroidogenic organs for subsequent synthesis of bile acids and lipoproteins and eventual elimination from the body (43, 44) and inhibiting the oxidation of LDL as well as the atherogenic effects of oxidized LDL by virtue of its antioxidant (45) and anti-inflammatory (44) properties.
Flavonoids are reported to increase HDL-C concentration and decrease in LDL and VLDL levels in hypercholesteremic rats (42). Cholesterol lowering effect of avocado seeds may also be attributed to the presence of beta sitosterol and tocopherols in the seeds. Beta sitosterol is a natural plant sterol which maintains healthy cholesterol levels. It does this by interfering with cholesterol absorption. Tocopherols are natural antioxidants that protect tissues from lipid peroxidation by mopping up free radicals, thus they prevent the oxidation of LDL receptors hence facilitating the uptake of cholesterol into tissues (46). In addition, avocados have been found to contain three times the amount of glutathione present in any other fruit (47). High antioxidant level may produce free radicals that could oxidize LDL receptors and reduce the uptake of cholesterol into cells; this could lead to increase in the level of plasma total cholesterol and LDL cholesterol. It has also been shown that reducing plasma level of LDL cholesterol sharply reduced the risk of coronary heart disease (48). This suggests that EAF of PA may possess antioxidant effects. This is most likely the case because isolation of bioactive phytoconstituents from the leaves of *Persea americana* has produced compounds with antioxidant properties such as luteolin, rutin, quercetin and apigenin (49).

Hyperlipidemia, a well known risk factor for cardiovascular disease, especially atherosclerotic coronary artery disease (CAD), is one of the major causes of premature death globally and it is expected to be the most important cause of mortality in India by the year 2010 (50). AIP is an easily available cardiovascular risk marker and a useful measure of response to pharmacological intervention (51). When the AIP value falls between -0.3 - 0.1, there is low cardiovascular risk; between 0.1 - 0.24 there is an intermediate risk, and greater than 0.24 there is high risk (31, 32). The AIP of the rats increased significantly (PS<0.05) in the olive oil group when compared to distilled water group. Also, fractions (EAF 250 mg/kg) significantly lowered the AIP of the rats when compared to distilled water. This could be as a result of modifications of the lipids by both atorvastatin and the fractions of *Persea americana*. Linoleic acid which is one of the most important unsaturated fatty acids in human food because of its prevention of distinct vascular heart disease (52) is found in avocado and could be the cause of low cardiovascular risk in some of the rats. Several studies revealed that an increase in HDL cholesterol and decrease in TC, LDL cholesterol and TG is associated with a decrease in the risk of ischemic heart diseases (53). Most of the antihyperlipidemic drugs are causing significant reduction in both TC and TG cholesterol levels (54). Hyperlipidemia-associated lipid disorders are considered to cause the atherosclerotic cardiovascular heart disease (55). The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease (56).

Glucose level was increased by olive oil significantly. Atorvastatin and EAF (500 mg/kg) decreased glucose level significantly. Drug-induced increase in glucose levels has been reported to be attributable to excess mobilization of fat from the adipose tissue to underutilization of glucose (57). Extract-
induced decrease in the concentration of blood glucose in the rats treated with the fractions may be the result of increased glycolysis. This is in agreement with the previous studies on \textit{Catharanthus} \textit{roseus} (58) and \textit{Tinospora} \textit{cordifolia} (59).

CONCLUSION
From the present work, it can be concluded that the ethyl acetate fraction of methanolic seed extract of \textit{Persia americana} does have a hypolipidemic effect and could serve as a good alternative for managing hyperlipidemia and also hyperlipidemic induced diabetes.

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Olive oil enriched diet effect on serum

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