Hypolipidemic effects of *Olax subscorpioidea* Oliv. root extract in experimental rat model

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ABSTRACT

*Olax subscorpioidea* (Olacaceae) is a major component of a recipe used traditionally in the management of Obesity. In view of the traditional therapeutic use of the plant, this research screened the ethanol root extract of the plant for hypolipidemic effect in Wistar rats. This was carried out to validate its potential as a lipid lowering agent. The experimental rats were randomly distributed into five groups of 7 animals each. Group 1 (not induced with hyperlipidemia) received distilled water; Group 2 (induced with hyperlipidemia). Group 3 (induced) received 10 mg/kg bw of atorvastatin. Groups 4 and 5 were induced with hyperlipidemia and treated with 200 and 400 mg/kg bw extract of *Olax subscorpioidea* respectively for 14 days. *Olax subscorpioidea* root contained saponins (865.00 mg/100 g), alkaloids (963.33 mg/100 g), tannins (863.33 mg/100 g), flavonoids (636.67 mg/100 g), anthraquinones (46.67 mg/100 g) and proanthocyanidins (2.67 mg/g). The extract gave 47.30% inhibition against DPPH+. Significant decrease in TC, TG and LDL was recorded in treated groups with 200 and 400 mg/kg bw of the extract. There was also a significant increase in HDL level of treated groups when compared with induced group. This result suggests that ethanol extract of *Olax subscorpioidea* at 200 and 400 mg/kg bw possesses hypolipidemic effects on diet induced hyperlipidemic rats. The observed bioactivity could be attributed to phytochemical components, proximate contents as well as antioxidant activity of the plant.

Keywords: *Olax subscorpioidea*, Phytochemicals, Nutrients, Antioxidant, Lipid profile, Biochemicals, Histopathology.

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INTRODUCTION

*Olax subscorpioidea* Oliv. belongs to the family of Olacaceae. It is widely distributed in Nigeria, Zaire and Senegal part of Africa (Ayandele and Adebiyi, 2007). It has many household names in Nigeria. For instance, it is called “Aziza” and “Ukpakon” in Eastern, “Ifo” in Western, and “Gwano kurmi” in Northern part of Nigeria respectively (Victoria *et al.*, 2010; Olowokudejo *et al.*, 2008; Ibrahim *et al.*, 2007). The plant has formed part of traditional recipe for the treatment of various diseases in Africa. It has been reported by indigenous people to serve as anti-asthma, anti-diabetes, anti-cancer, anti-rheumatism and anti-typhoid ((Adeoluwa *et al.*, 2015; Adeoluwa *et al.*, 2014).

Hyperlipidemia is a lipoprotein metabolic disorder characterized by high serum low density lipoprotein (LDL) and low serum high density lipoprotein (HDL). It is a condition of excess fatty substances called lipids, largely cholesterol and triglycerides, in the blood (Kelly, 2010; Kishor *et al.*, 2007). Hyperlipidemia plays a major role in the development of cardiovascular diseases in Africa (Yusuf *et al.*, 2001). The prevalence of hyperlipidemia was estimated at 16.1%; obesity was estimated at 6.5%; while the cardiovascular diseases (CVD) accounted for 12% of all deaths in Nigeria (WHO, 2011; Yekeen *et al.*, 2003). All these were due to increasing intake of cholesterol rich foods, overweight, alcohol abuse, age and stress (Sani *et al.*, 2010; Salim *et al.*, 2001). Presently, orthodox drugs such as statins, nicotinic acid derivatives, fibric acid derivatives, bile acid binding resins and cholesterol absorption inhibitors have been associated with side effects such as itching, constipation, stomach upset, nausea, vomiting, headache and dizziness (Koh *et al.*, 2008; Davidson, 2001). In view of the side effects of orthodox drugs, the prevalence of hyperlipidemia and the
Hypolipidemic effects of Olax subscorpioidea

importance of medicinal plants towards disease management, there is need to discover new plants for use in combating cholesterolemic related diseases (Nordestgard et al., 2010; Minto and Blacklock, 2008).

MATERIALS AND METHODS

Collection, identification and preparation of plant materials: Fresh roots of Olax subscorpioidea were collected from Botanical Garden, University of Ibadan. The plant sample was identified and deposited at University of Ibadan Herbarium (UIH) with voucher number UIH 22549. The plant material was powdered and stored in airtight bottles prior to experiment. The powdered sample (400 g) was extracted in 1.6 liter of ethanol (50%) using cold maceration. Thereafter, the solution was filtered with whatman 1 filter paper. The filtrate was concentrated using distillation method (Trease and Evans, 2003) and the crude extract obtained was stored at 4°C.

Chemicals and Experimental materials: Atorvastatin tablets (10 mg); 10% formalin; glacial acetic acid; ethanol (50%); meyer’s reagent. Feeds; distilled water; cotton wool; hand gloves; light microscope; syringe and cannula; spectrophotometer; lithium heparin bottles; weighing balance; foil paper, feeders and drinkers; measuring cylinder and centrifuge.

Phytochemical analysis: The powdered sample was screened for the presence of secondary metabolites using methods described by AOAC (2005).

Proximate analysis: The proximate analysis of the powdered sample was carried out for the moisture, ash, crude fat, protein, crude fibre, carbohydrate contents using AOAC (2005) guidelines.

Antioxidant analysis: The radical scavenging ability of the sample was determined according to the method of Mensor et al. (2001).

Ethical approval: This study was conducted in accordance with the Ethical Committee Guidelines of University of Ibadan on the use of animals for research (UI-ACUREC/APP/2015/016).

Experimental animals and Acclimatization: Thirty-five (35) male Wistar rats of average weight of 69.84 ± 2.59g were used for the experiment. The animals for the research were procured at the animal house, Department of Anatomy, University of Ibadan. They were acclimatized for 2 weeks in the animal house of Faculty of Veterinary Medicine, University of Ibadan. The animals were fed and water was supplied ad libitum. They were exposed to alternate cycle of 12hrs of darkness and 12hrs light. All experiments were performed according to guidelines for care of laboratory animals and the ethical guidelines (National Institute of Health, 1992).

Grouping of experimental animals: The animals were divided into 5 groups comprised of 7 rats each after 2 weeks of acclimatization.

Group 1: Positive control (not induced with hypercholesterolemia)
Group 2- Negative control (induced with hypercholesterolemia, not treated)
Group 3- Treatment group orally (with 10 mg/kg bw/day of Atorvastatin)
Group 4- Treatment group orally (with 200 mg/kg bw/day extract of Olax subscorpioidea)
Group 5- Treatment group orally (with 400 mg/kg bw/day extract of Olax subscorpioidea)

Induction of Hyperlipidemia: Induction of high fat was done using the method by Matos et al. (2005).

Dosage calculation of crude extracts and standard drug administered on rats: The dosage calculation of crude plant extract and standard drug for the experiment was done using OECD’s guidelines (2000).

Baseline analysis of lipid profile of rats after induction of Hyperlipidemia: Baseline lipid profile of both the positive control (not induced) and negative control (induced) was examined using method of IACUC guidelines (2002) with little modification.

Toxicity studies of extracts before treatment: The acute toxicity of the extract was evaluated using method by Lorke (1983).

Administration of plant extracts and standard drug (Atorvastatin): Administration of the plant extracts and reference drug were done using guideline by OECD (2000). Doses of 200 mg/kg and 400mg/kg body weight of extracts of Olax subscorpioidea were selected from the analysis on toxicity studies of the ethanolic root extract of the plants in rats. They were administered into the experimental rats orally for 14 days.

Collection of blood samples after two weeks of treatment: The collection of blood was done using IACUC guidelines (2002) by retro orbital sampling method.

Lipid profile analysis: The plasma samples were analyzed for serum total cholesterol (TC), triglycerides (TG) and high density lipoprotein (HDL). This was determined using randox laboratories kit based on enzymatic end point method (Vogel et al., 2002). The Low-density lipoprotein cholesterol (LDL) was calculated using Friedewald formula.

Biochemical analysis: The biochemical parameters were determined using standard biochemical kit. Parameters assayed include total proteins, albumins, globulins, aspartate amino transferase (AST), alanine amino transferase (ALT) and bilirubins (Kutty and Jacob, 2004).

Histopathological studies: The histopathological analysis of heart and liver were done using method by Morton et al. (1997).

Statistical analysis: Data were analysed using one-way analysis of variance (ANOVA) and values were expressed as mean ± SEM. The Duncan multiple range test (DMRT) was used to test the means for significance (P <0.05).
RESULTS

The phytochemical screening showed that *O. subscorpioidea* root has alkaloid, saponins, low tannins together with low anthraquinones and very low Proanthocyanidins contents, respectively (Table 1). Table 2 showed the proximate contents of the sample in this order: Carbohydrates > Crude proteins > Crude fibers > Moisture contents > Crude ash > Crude fats. This therefore shows that *O. subscorpioidea* root has high carbohydrate contents and low crude fats (Table 2).

**Table 1:**
Phytochemical components of *Olax subscorpioidea* root

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Composition (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>963.33 ± 7.26a</td>
</tr>
<tr>
<td>Saponins</td>
<td>865.00 ± 2.89b</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>636.67 ± 7.26c</td>
</tr>
<tr>
<td>Tannins</td>
<td>863.33 ± 7.26b</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>ND</td>
</tr>
<tr>
<td>Anthraquinones</td>
<td>46.67 ± 1.67d</td>
</tr>
<tr>
<td>Proanthocyanidins</td>
<td>2.67 ± 0.44e</td>
</tr>
</tbody>
</table>

Values are expressed in Mean ± SEM; n= 3; Values with different alphabetical superscript in a column are significantly different at p <0.05.

**Table 2:**
Proximate composition of *Olax subscorpioidea* root

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude proteins</td>
<td>21.03 ± 0.99b</td>
</tr>
<tr>
<td>Crude fibers</td>
<td>12.73 ± 0.03c</td>
</tr>
<tr>
<td>Crude fats</td>
<td>2.37 ± 0.09d</td>
</tr>
<tr>
<td>Moisture contents</td>
<td>8.73 ± 0.03d</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>49.03 ± 0.03a</td>
</tr>
<tr>
<td>Crude ash</td>
<td>6.10 ± 0.06e</td>
</tr>
</tbody>
</table>

Values are expressed in Mean ± SEM; n= 3; Values with different alphabetical superscript in a column are significantly different at p <0.05.

**Table 3:**
Polyphenol content and Antioxidant activity of *Olax subscorpioidea* root

<table>
<thead>
<tr>
<th>Polyphenol content</th>
<th>% inhibition against DPPH*</th>
</tr>
</thead>
<tbody>
<tr>
<td>38.77± 0.15 mg GAE/g</td>
<td>47.30 ± 0.06</td>
</tr>
</tbody>
</table>

Values are expressed in Mean ± SEM; n= 3; GAE = Gallic Acid Equivalent.

In another experiment, the antioxidant capacity of the sample showed high polyphenol content (38.77 GAE/g) and percentage inhibition against DPPH* radicals (47.30% inhibition) as indicated in Table 3. The results of the baseline lipid profile showed a significant increase in TC (193.20 ± 20.13), TG (127.20 ± 18.35), HDL (62.40 ± 1.75) and LDL (87.80 ± 23.22) in hyperlipidemia group when compared with control rats TC (95.20 ± 11.08), TG (55.60 ± 7.65), HDL (47.20 ± 2.42) and LDL (36.80 ± 11.00), respectively as shown in Table 4. The lipid profile after the induction of hyperlipidaemia together with different treatment groups showed that *O. subscorpioidea* administration significantly reduced TC, TG and LDL-cholesterol (Table 5). On the other hand, serum HDL-cholesterol was however increased significantly in rats administered with extract and standard drug (Atorvastatin) when compared the hyperlipidaemic untreated rats (Table 5). Our data showed significant increase in serum bilirubin and significant reduction in total protein, serum albumin and globulin in hyperlipidaemic untreated rats when compared to the control and other treatment groups (Table 6).

**Table 4:**
Baseline lipid profile of the rats after 5 weeks of induction of hyperlipidemia

<table>
<thead>
<tr>
<th>Lipid profile (mg/dl)</th>
<th>Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>TC</td>
<td>95.20 ± 11.08a</td>
</tr>
<tr>
<td>TG</td>
<td>55.60 ± 7.65b</td>
</tr>
<tr>
<td>HDL</td>
<td>47.20 ± 2.42b</td>
</tr>
<tr>
<td>LDL</td>
<td>36.80 ± 11.00b</td>
</tr>
</tbody>
</table>

Values are expressed in Mean ± SEM, n= 5; Values with different alphabetical superscript in a column are significantly different at p<0.05. TC= Total Cholesterol; TG= Triglyceride; HDL= High Density Lipoproteins; LDL= Low Density Lipoproteins.

**Table 5:**
Lipid profile of experimental rats after 2 weeks of treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>Lipid Profile (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TC</td>
</tr>
<tr>
<td>1 (control)</td>
<td>90.48 ± 4.99bc</td>
</tr>
<tr>
<td>2 (Induced)</td>
<td>122.20 ± 6.54a</td>
</tr>
<tr>
<td>3 (10 mg ATV)</td>
<td>81.20 ± 6.47c</td>
</tr>
<tr>
<td>4 (200 mg sample)</td>
<td>99.76 ± 3.10bc</td>
</tr>
<tr>
<td>5 (400 mg sample)</td>
<td>112.92 ± 4.13ab</td>
</tr>
</tbody>
</table>

Values are expressed in Mean ± SEM, n= 5; Values with different alphabetical superscript in a column are significantly different at p<0.05. TC= Total Cholesterol; TG= Triglyceride; HDL= High Density Lipoproteins; LDL= Low Density Lipoproteins; ATV= Atorvastatin.
Table 6:
Effects of samples on the biochemical parameters of rats after 2 weeks of treatment

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>TP</td>
</tr>
<tr>
<td>1 (control)</td>
<td>7.83±0.33&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 (Induced)</td>
<td>7.83±0.17&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>3 (10 mg ATV)</td>
<td>8.17±0.33&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>4 (200 mg O.S)</td>
<td>8.33±0.17&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>5 (400 mg O.S)</td>
<td>8.83±0.03&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed in Mean ± SEM, n= 5, Values with different alphabetical superscript in a column are significantly different at p<0.05. TP- Total proteins, AL- Albumins, GL- Globulins, BL- Bilirubin, AST-Aspartate amino transferase, ALT- Alanine amino transferase.

The photomicrograph of the cardiac tissues showed moderate congestion of coronary blood vessels of hyperlipidaemic untreated rats whereas in rats administered with O. subscorpioidea extract and standard hypolipidaemic agent (Atorvastatin); there were areas of moderate congestion of coronary blood vessels and cardiomyocytes contain large cytoplasmic vacuole, respectively (Plate 1). In Plate 2, the hepatocytes showed no visible lesions in the control rats. However, there are areas of vacuolar degeneration and Kupffer cell hyperplasia in rats treated with O. subscorpioidea extract and Atorvastatin.

DISCUSSION

The phytochemical composition of O. subscorpioidea root recorded in this study is in line with previous reports (Adegbite et al., 2015; Ayandele and Adebiiyi, 2007). The absence of cardiac glycosides in O subscorpioidea follows the previous report by Adegbite et al. (2015). Saponins have been reported to have beneficial effects on blood cholesterol levels. They bind with bile salt and cholesterol in the intestinal tract (Oakenfull and Sidhu, 1990). Consumption of food rich in tannins helps in protection of vascular system.
They do this by strengthening the tiny capillaries that carry oxygen and essential nutrients to all cells (Chang et al., 2001). Flavonoids, proanthocyanidins, anthraquinones in a plant have been implicated to play significant roles in the metabolism of lipids by blocking the angiotensin and convert enzymes that raise blood pressure (Ngamukote et al., 2011; Abolaji et al., 2007).

The result of nutritional content of O. subscorpioidea agrees with the findings of other authors (Ibukunoluwa et al., 2015; Osuntokun and Oluwafolose, 2015; Otori and Mann, 2014). Dietary soluble fiber in plants plays a crucial role in lowering lipids (Brown et al., 1998). They are probably related to decrease dietary cholesterol absorption, increase primary bile acid synthesis. Presence of low moisture in plant has been reported as an important factor that could hinder the growth of micro organisms on plant material and promote shelf-life of the plant (Erukainure et al., 2011; Adeyeye and Ayejuyo, 1994). Diet rich in protein contributes to the formation of hormones which controls the variety of body functions such as growth repairs and maintenance of body protein (Carnovale et al., 1991). Ash content is useful in assessing the quality grading of plants and also gives an idea of the amount of mineral element present in such plant (Smart, 1996). The ash content in plants also implies that it is very nourishing and suitable for consumption. Carbohydrates provide the energy required for normal physiological functions; they help to power cells and tissues in the body (Ramalingam, 2010).

The results on polyphenol contents and antioxidant activity of O. subscorpioidea against DPPH+ are in line with past reports by authors (Womeni et al., 2013; Konan et al., 2013). Polyphenol compounds have been reported to be crucial for bioactivities of plants (Nagavani et al., 2010). They exhibit a wide spectrum of medicinal properties, such as anti-cancer, anti-allergic and are cardio-protective (Konan et al., 2013; Banerjee and Bonde, 2011). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1999). The acute toxicity study of O. subscorpioidea in this study corroborates the reports of other authors (Adebayo et al., 2014; Victoria et al., 2010). The significance increase in baseline lipid profile of hyperlipidaemic rats in this study is similar to the result of Sowmya and Ananthi (2011) on Mimosa pudica. The importance of baseline lipid profile assessment in animals has been reported as an indicator for hyperlipidemia (Abolaji et al., 2007; Yakubu et al., 2003). Decrease in the level of bilirubin and ALT of treated groups recorded in this study is in line with report by Adebayo et al. (2014). Several reports have revealed the essentiality of biochemical parameters (AST, ALT, albumin, total bilirubin and globulin concentration) as an important factor in examining the functioning of organs in body (Amresh et al., 2008; Guyton et al., 2008). The observation on the histopathology of the liver and heart of rats treated with Olax subscorpioidea agree with those of Adebayo et al. (2014).
Injac et al. (2008) explained that the increase in the serum enzyme levels help in contributing to increased leakage from damaged and necrotic hepatocytes preventing the heart and other organs from atherosclerosis.

In conclusion, this study suggests that ethanol extract of Olax subscorpioidea root at 200 and 400 mg/kg bw possesses hypolipidemic effects on diet induced hyperlipidemic rats. The observed bioactivity could be attributed to phytochemical and proximate components, as well as antioxidant activity of the plant.

REFERENCES


