Effect of Aqueous Whole Plant Extract of Selaginella myosurus on Lipid Profile of Wistar Rats

Omeodu S. I., Peters D. E.*, Oki J.

Department of Biochemistry, Faculty of Science, University of Port Harcourt, Rivers State, Nigeria

Email address
petersdikioye@ymail.com (Peters D. E.), dikioye.peters@uniport.edu.com (Peters D. E.)

Citation

Abstract
Cardiovascular disease continues to be the leading cause of death across the globe today. Treatment with synthetic drugs is associated with several adverse effects. This study was designed to investigate the effect of aqueous whole plant extract of Selaginella myosurus on lipid profile of wistar rats. A total of thirty six (36) wistar rats of both sexes weighing between 100.5g-149.5g were divided into nine groups of four rats each. Group 1 received distilled water, while groups 2-5 and 6-9 received 400, 600, 800, and 1000mg/kg BW of extract for 7 and 14 days respectively. Rats were sacrificed 24 hours after the last treatment and blood samples collected for determination of total cholesterol (T-CHOL), triglyceride (TG), high density lipoprotein-cholesterol (HDL-CHOL) and low density lipoprotein-cholesterol (LDL-CHOL), histological investigation on the heart tissue and phytochemical screening of the plant. Significant reductions (p<0.05) were observed in T-CHOL and TG in all the extract-treated groups when compared to control value and non significant reduction (p>0.05) HDL-CHOL and LDL-CHOL in all the groups except in groups 3 and 5 respectively. Phytochemical screening of plant revealed the presence of flavonoids, triterpenoids, saponins, tannin, steroid, cardiac glycoside and phenol in decreasing order (3 2.19±0.23, 26.24±0.12, 23.74±0.20, 18.74±0.17, 16.53±0.12, 15.28±0.23 and 13.10±0.11 mg/100g respectively).Histological result revealed that all heart muscles were in good histological conditions in control and all extract treated groups. Hence, aqueous whole plant extract of Selaginella myosurus has hypolipidemic effect, could protect the heart against cardiovascular disease (CVD) and hence a promising potential herbal pharmaceutical agent.

1. Introduction
Cardiovascular disease continues to be the leading cause of death across the globe today the major ones being coronary heart diseases, stroke and hypertension [1]. Elevated plasma lipids are risk factors in cardiovascular problems. Hyperlipidemia and other abnormal blood lipid profile are largely of genetic origin or due to unwholesome nutritional habits. Lipids and other substances accumulates on arterial wall, forming plaque, which occlude the vascular lumen and obstruct the blood flow to vital organs such as the heart, brain, liver or kidney. Obstruction of blood supply to the heart, brain, liver or kidney cause coronary heart diseases, stroke or kidney failure.

Hyperlipidemia refers to elevated levels of lipids and cholesterol in the blood, and is also identified as dyslipidemia, to describe the manifestations of different disorders of lipoprotein metabolism. Although elevated low density lipoprotein cholesterol (LDL) is
thought to be the best indicator of atherosclerosis risk, dyslipidemia can also be described as elevated total cholesterol (TC) or triglycerides (TG), or low levels of high density lipoprotein cholesterol (HDL).

High level of blood cholesterol is a contributory factor of atherosclerosis and many lipid associated ailments like obesity, heart attacks and stroke and kidney failure.[2] have shown that lipid associated disorders are not only attributed to the total serum cholesterol, but also to its distribution among different lipoproteins. The low density lipoproteins (LDLs) are the major carriers of cholesterol towards tissues having atherogenic potential, while the high density lipoproteins (HDLs) carry cholesterol from peripheral tissues to the liver [3]. HDLs thus give protection against many cardiac problems and obesity [4]. Although genetic factors recline behind these lipid disorders, in most of the cases it is allied with diets high in saturated fats or trans fats.

The clinical consequences of these disease conditions are serious and meaningful research efforts to improve the knowledge and understanding of the pathogenesis is essential, in order to provide a more rational approach to their prophylaxis and treatment. [5-6].

Selaginellamyosurus Alston with an alternative name of Stachygynandrum, wide spread in all continents predominantly terrestrial plant of lowland to mid-montane primarily rainforest but preferring more open glades and river banks and therefore a frequent component of secondary forest in these areas. Selaginella myosurus (Sw.) Alston (Lycopodiummyosurus Sw.: Selaginella scandens P. Beauv.; Stachygynandrumscandens P. Beauv.) are creeping or ascendant plants with simple, scale-like leaves (microphylls) on branching stems from which roots also arise. It appears that the traditional use of Selaginella myosurus in Nigeria and other part of West Africa is still relatively rare, compared to the number of species that are grown in this region. It is commonly referred to by indigenes of Rivers State as Akoro, ukor. It is a powerful plant that is used in many regions due to different beliefs, it is found in swamp forests, disturbed areas, along roadsides, edge of forests.

Selaginella is traditionally used to treat several diseases such as injury, treatment of post childbirth, cancer, skin disease, headaches, fever, respiratory infections, urinary tract infections, menstrual disorders, liver disorders, fractures and arthritis. All parts of the plant can be used, although sometimes they are called only a leave (herb) [7].

Its use can be solely or in combination, fresh or dried, eaten immediately or cooked. The plant sweet and have warm effects [8].

Selaginella contains a variety of secondary metabolites such as alkaloids, phenol (flavonoids, tannins, and saponins) and terpenoids [9]. The main secondary metabolite of this plant is bioflavonoid, whose type is varied depending on the species. Bioflavonoid, once known as “Vitamin P” is useful in treatment and prevention of many health conditions. It is referred to as “nature’s biological response modifiers” modify body’s reaction to compounds such as allergens, viruses and carcinogens. These compounds act as antioxidants, anti-inflammatory, antimicrobial, antifungal, antiviral etc. [10]. It is well known that plants generally contain secondary metabolites and some of these secondary metabolites have been shown to be highly biologically active [11] and as well as exhibiting physiological activity [12]. Saponins cause hypocholesterolemia by binding cholesterol, making it unavailable for absorption [13].

Flavonoids are a group of phytochemicals found in varying amounts in foods and medicinal plants which have been shown to exert potent anti-oxidant activity against the superoxide radical [14]. Its consumption has been documented not to be associated with mortality due to coronary heart disease. This may be as a result of its antioxidant activity and subsequent inhibitions of Low Density Lipoproteins (LDL) oxidation known to have been attributed to the dietary and supplemental intake of flavonoids and other micronutrients. Tannins hasten the healing of wounds and inflamed mucous membrane [15]. The lowering level of serum cholesterol using diet or drugs decreases the incidence of coronary heart disease [16, 17]. Steroids are a group of cholesterol derived lipopholic, low-molecular weight compounds and can be grouped on the basis of type of organism in which they are present. Steroids are classified into three broad categories; insect steroids, vertebrate steroids and plant steroids. Plant steroids are of two broad categories: phytosterols and brassinosteroids. Phytosterols covers both plant sterols and stanols [18]. They are plant components with a structure similar to cholesterol [19], although they are more poorly absorbed by the intestine. They are classified into different groups depending on their structure and biosynthesis [20]. Their exact mechanism of action and cholesterol lowering properties are not known, but, because their structure is similar to that of cholesterol, they compete for solubilization in the micelles and therefore inhibit intestinal absorption of both dietary and endogenous cholesterol [21] lowering postprandial cholesterol level in the blood.

2. Materials and Methods

2.1. Apparatus/Equipment

Spectrophotometer (BSA 3000), SFRI France,Rotary evaporator, Centrifuge (Universal laboratory century), Hettich Zentrifugen, Metlar weighing balance, SIEMENS Advia 2120 Automated Analyzer.

2.2. Reagents/Chemicals

All reagents and chemicals are of analytical grade.

2.3. Collection/Identification of Plant

Selaginella myosurus was collected in the surrounding bush of the University of Port Harcourt in Choba community of Ohio/Akpor Local Government Area of Rivers state. A voucher specimen (UPH-NO.C-129) was authenticated by a
2.4 Extract Preparation

The whole plant of Selaginella myosurus was washed with running tap water and air dried for 2 weeks before grinding into powdered form. The coarsely powdered plant material was macerated in a maceration jar for 24 hours, with distilled water. Filtration was done using Whatman filter paper in a glass funnel placed in a retort stand. The filtrate was allowed for about 1-2 hours to observe any residue or sediment. After having a clear filtrate, it was put in a rotary evaporator which separated the water from the extract, leaving the extract in a paste form. The extract was then poured into a crucible plate for drying on a steam bath at 40°C to 50°C. The crude extract was stored in a refrigerator pending usage.

2.5 Phytochemical Screening

Phytochemical screening of the whole plant of Selaginella myosurus was done using standard procedure as described by [22] in the Department of Pharmacognosy, Faculty of Pharmacy, University of Port Harcourt.

2.6 Source of Animals

A total of thirty six (36) wistar rats of both sexes weighing between 100.5g-149.5g were purchased from an animal breeding facility in Choba community, and were kept in the Department of Biochemistry, University of Port Harcourt Animal House, Choba park for one week acclimatization. The rats were fed with normal rat feed and water ad libitum.

2.7 Lethal Dose (LD₅₀) Determination

LD₅₀ determination was done using an "up-and-down" procedure described by [23]. Three doses of 1000mg/kg, 3000mg/kg, and 5000mg/kg were orally administered to 3 groups of rats (n=2 rats per group). The rats were observed for 24 hours and for a period of 1 week. No death was recorded; therefore, safe doses of 400, 600, 800 and 1000mg/kgBW were selected.

2.8 Experimental Design

The rats were divided into nine (9) groups (n=4 rats).

GROUP 1 (Control): 0.5ml of distilled water was orally given to the animals in this group daily for 14 days.

GROUP 2 (400mg/kg b.w extract): A single daily dose of 400mg/kg b.w of aqueous whole plant extract of Selaginella myosurus was orally administered to rats in this group for 7 days.

GROUP 3 (600mg/kg b.w extract): A single daily dose of 600mg/kg b.w of aqueous whole plant extract of Selaginella myosurus was orally administered to rats in this group for 7 days.

GROUP 4 (800mg/kg b.w extract): A single daily dose of 800mg/kg b.w of aqueous whole plant extract of Selaginella myosurus was orally administered to rats in this group for 7 days.

GROUP 5 (1000mg/kg b.w extract): A single daily dose of 1000mg/kg b.w of aqueous whole plant extract of Selaginella myosurus was orally administered to rats in this group for 7 days.

GROUP 6 (400mg/kg b.w extract): A single daily dose of 400mg/kg b.w of aqueous whole plant extract of Selaginella myosurus was orally administered to rats in this group for 14 days.

GROUP 7 (600mg/kg b.w extract): A single daily dose of 600mg/kg b.w of aqueous whole plant extract of Selaginella myosurus was orally administered to rats in this group for 7 days.

GROUP 8 (800mg/kg b.w extract): A single daily dose of 800mg/kg b.w of aqueous whole plant extract of Selaginella myosurus was orally administered to rats in this group for 7 days.

GROUP 9 (1000mg/kg b.w extract): A single daily dose of 1000mg/kg b.w of aqueous whole plant extract of Selaginella myosurus was orally administered to rats in this group for 7 days.

Sacrifice, Collection and Preparation of Plasma

At the end of 7 and 14 days, all the animals were anaesthetized with chloroform before decapitated for collection of blood. The blood was stored in heparinised sample bottle, spun at 5000rpm using MSE centrifuge to obtain plasma for biochemical investigations.

3. Biochemical Investigation

Total Cholesterol (TC) and Triacylglycerol (TG), were estimated by enzymatic methods described by [24] using assay kits (Randox Laboratories Ltd, UK). High-Density Lipoprotein Cholesterol (HDL-C) was determined by enzymatic method described by [25] using assay kits (Randox Laboratories Ltd, UK). Low-Density Lipoprotein Cholesterol (LDL-C) was calculated using the formular by [26].

3.1 Histopathological Studies

The rats were dissected using a set of dissection kit and hearts from control and extract treated groups were collected and fixed in 10% freshly prepared formalin for 48 hours and subsequently dehydrated in alcohol, cleared with xylol and embedded in paraffin wax. Sections of lobe at about 5μm were mounted on glass slides and stained with haematoxylin and eosin [27].

3.2 Statistical Analysis

All the values were reported as mean ± standard error of mean (M ± SEM). Statistical analysis was performed using SPSS version 20.0 (IBM, U.S.A). The data were analyzed using one-way analysis of variance (ANOVA) and significant difference were determined using post hoc Turkey’s test for multiple comparisons at p< 0.05.
4. Result

Table 1. Effect of Aqueous Whole Plant Extract of Selaginella myosurus on Lipid Profile of Wistar Rats.

<table>
<thead>
<tr>
<th>TREATMENT GROUPS (Mg/kgBW)</th>
<th>PARAMETERS</th>
<th>TG (umol/L)</th>
<th>CHOL (umol/L)</th>
<th>HDL-CHOL (umol/L)</th>
<th>LDL (umol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>WATER CONTROL</td>
<td></td>
<td>1.65±0.07*</td>
<td>3.40±0.08*</td>
<td>1.08±0.05*</td>
<td>1.53±0.10*</td>
</tr>
<tr>
<td>400mg/kgBW EXT FOR 7 DAYS</td>
<td></td>
<td>1.38±0.05*</td>
<td>2.73±0.14*</td>
<td>0.93±0.05</td>
<td>1.15±0.16</td>
</tr>
<tr>
<td>600mg/kgBW EXT FOR 7 DAYS</td>
<td></td>
<td>1.33±0.05*</td>
<td>2.73±0.15*</td>
<td>0.83±0.05</td>
<td>1.25±0.10</td>
</tr>
<tr>
<td>800mg/kgBW EXT FOR 7 DAYS</td>
<td></td>
<td>1.40±0.04*</td>
<td>2.73±0.08*</td>
<td>0.85±0.06</td>
<td>1.20±0.07</td>
</tr>
<tr>
<td>1000mg/kgBW EXT FOR 7 DAYS</td>
<td></td>
<td>1.28±0.05*</td>
<td>2.45±0.06*</td>
<td>0.90±0.04</td>
<td>0.95±0.5*</td>
</tr>
<tr>
<td>400mg/kgBW EXT FOR 14 DAYS</td>
<td></td>
<td>1.48±0.48</td>
<td>3.05±0.06</td>
<td>1.03±0.05</td>
<td>1.30±0.09</td>
</tr>
<tr>
<td>600mg/kgBW EXT FOR 14 DAYS</td>
<td></td>
<td>1.35±0.03*</td>
<td>2.85±0.06*</td>
<td>0.90±0.04</td>
<td>1.30±0.08</td>
</tr>
<tr>
<td>800mg/kgBW EXT FOR 14 DAYS</td>
<td></td>
<td>1.38±0.05*</td>
<td>2.78±0.09*</td>
<td>0.88±0.05</td>
<td>1.18±0.05</td>
</tr>
<tr>
<td>1000mg/kgBW EXT FOR 14 DAYS</td>
<td></td>
<td>1.33±0.05*</td>
<td>2.68±0.09*</td>
<td>0.88±0.05</td>
<td>1.13±0.09</td>
</tr>
</tbody>
</table>

Data are represented in Mean±Standard Error of Mean (M±SEM)
Similar superscripts represent significant different (p<0.05) in the same row

Table 2. Qualitative Phytochemical Screening of Whole Plant Extract of Selaginella myosurus.

<table>
<thead>
<tr>
<th>SECONDARY METABOLITES</th>
<th>TEST</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Drangedorff</td>
<td>-ve</td>
</tr>
<tr>
<td></td>
<td>Mayer</td>
<td>-ve</td>
</tr>
<tr>
<td></td>
<td>Hager</td>
<td>-ve</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Lead acetate</td>
<td>-ve</td>
</tr>
<tr>
<td></td>
<td>Alkali</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>FeCl₃</td>
<td>+ve</td>
</tr>
<tr>
<td>Tannins</td>
<td>Phlobatannins</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Gelatin</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>ND</td>
</tr>
<tr>
<td>Anthraquinone</td>
<td>Free Anthraquinone</td>
<td>-ve</td>
</tr>
<tr>
<td></td>
<td>Combined Anthraquinone</td>
<td>-ve</td>
</tr>
<tr>
<td></td>
<td>Liebermann-Buchard</td>
<td>+ve</td>
</tr>
<tr>
<td>Triterpenoid/steroids</td>
<td>Salwoski</td>
<td>+ve</td>
</tr>
<tr>
<td>Fixed oil</td>
<td></td>
<td>+ve</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Molisch</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Fehlings</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Keller Killani</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Keilde</td>
<td>+ve</td>
</tr>
<tr>
<td>Cyanogenic glycosides</td>
<td>Frothing</td>
<td>-ve</td>
</tr>
<tr>
<td>Saponins</td>
<td>Frothing</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>Haemolysis</td>
<td>-ve</td>
</tr>
<tr>
<td></td>
<td>Emulsion</td>
<td>+ve</td>
</tr>
</tbody>
</table>

Note:+Ve means present, -ve means absent, while ND is Not Determined

Table 3. Quantitative Photochemical Screening of Whole Plant of Selaginella myosorus.

<table>
<thead>
<tr>
<th>SECONDARY METABOLITES</th>
<th>mean± standard error of mean (M±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoid</td>
<td>32.19± 0.23</td>
</tr>
<tr>
<td>Saponins</td>
<td>23.74± 0.20</td>
</tr>
<tr>
<td>Cardiac glycoside</td>
<td>15.28± 0.23</td>
</tr>
<tr>
<td>Steroid</td>
<td>16.53± 0.12</td>
</tr>
<tr>
<td>Terpenoid</td>
<td>26.24± 0.12</td>
</tr>
<tr>
<td>Tannin</td>
<td>18.74± 0.17</td>
</tr>
<tr>
<td>Phenol</td>
<td>13.10± 0.11</td>
</tr>
</tbody>
</table>

HISTOLOGICAL EXAMINATION OF HEART SECTIONS OF RATS TREATED WITH DISTILLED WATER AND VARYING CONCENTRATIONS OF AQUEOUS WHOLE PLANT EXTRACT OF SELAGINELLA MYOSURUS FOR 7 AND 14 DAYS

Fig. 1. Heart section of control rat showing good histological conditions (H&E, x 400).

Fig. 2. Heart section of rat treated with 600mg/kgbw extract for 7 days showing good histological conditions (H&E, x 400).
Fig. 3. Heart section of rat treated with 800mg/kgbw extract for 7 days showing good histological conditions (H&E, x 400).

Fig. 4. Heart section of rat treated with 1000mg/kgbw extract for 7 days showing good histological conditions (H&E, x 400).

Fig. 5. Heart section of rat treated with 600mg/kgbw extract for 14 days showing good histological conditions (H&E, x 400).

Fig. 6. Heart section of rat treated with 800mg/kgbw extract for 14 days showing good histological conditions (H&E, x 400).

Fig. 7. Heart section of rat treated with 1000mg/kgbw extract for 14 days showing good histological conditions (H&E, x 400).

5. Discussion and Conclusion

It is widely accepted that a plant based diet with high intake of fruits and vegetables may reduce the risk of oxidative stress-related diseases such as cancer and cardiovascular diseases. Most bioactive food constituents are derived from plants and are collectively called phytochemicals. The majority of these phytochemicals are antioxidants. Antioxidants are defined as compounds that can delay, inhibit, or prevent the oxidation of oxidizable materials by scavenging free radicals and diminishing oxidative stress. Oxidative stress is an imbalanced state where excess quantities of reactive oxygen and/or nitrogen species (ROS/RNS, e.g., superoxide anion, hydrogen peroxide,
hydroxyl radical, peroxynitrite) overcome endogenous antioxidant capacity, leading to oxidation of a varieties of biomacromolecules, such as enzymes, proteins, DNA and lipids. Oxidative stress is important in the development of chronic degenerative diseases including coronary heart disease, cancer and aging[28]. Significant reductions (p<0.05) were observed in T-CHOL and TG concentrations in all the extract treated groups when compared to control value and non significant reduction (p>0.05) in HDL-CHOL and LDL-CHOL concentrations in all the groups except in groups 3 and 5 respectively in Table 1. Atherogenicity develops when LDL cholesterol, triacylglycerols and total cholesterol are elevated relative to plasma HDL-C[29]. Although elevated low density lipoprotein cholesterol (LDL) is thought to be the best indicator of atherosclerosis risk, [30] dyslipidemia can also be describe as elevated total cholesterol (TC) or triglycerides(TG), or low levels of high density lipoprotein cholesterol (HDL). Hence the plasma lipid profile lowering effect of the extract indicates protection against atherosclerotic cardiovascular disease (ASCVD) which includes stroke, peripheral arterial disease and coronary heart disease.

Phytochemical screening of plant from Tables 2 and 3 revealed the presence of flavonoids, triterpenoids, saponins, tannin, steroid, cardiac glycoside and phenol in decreasing order (32.19± 0.23, 26.24± 0.12, 23.74± 0.20, 18.74± 0.17, 16.53± 0.12, 15.28± 0.23 and 13.10± 0.11 mg/100g respectively). It is widely accepted that a plant based diet results of its antioxidant activity and subsequent inhibitions of Low Density Lipoproteins (LDL) oxidation known to have been attributed to the dietary and supplemental intake of flavonoids and other micronutrients.

Figures 1-7 showing histological results revealed that all the heart muscles were in good histological conditions both in the control and all extract treated groups. Hence, aqueous whole plant extract of *Selaginella myosurus* has hypolipidemic effect and could protect the heart against atherosclerotic cardiovascular disease (ASCVD), hence would benovel herbal pharmaceutical agent.

References


