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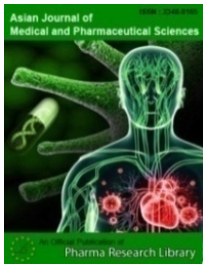

R E S E A R C H A R T I C L E

In Search of Natural Antihypertensives- Antihyperlipidaemic Potentials of Ethanolic Leaf Extract of *Diodia sarmentosa* on High Fat Diet-Fed Wistar Rats

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A B S T R A C T

Hypertension remains one of the non-communicable diseases of public health importance in the present millennium. Towards the search for this problem**,** and given the relationship between hyperlipidaemia and hypertension, this study investigated the antihyperlipidaemic potentials of ethanolic leaf extract of *Diodia sarmentosa* on high fat diet-fed wistar rats. Thirty (30) male wistar rats (150g-200g) were divided into five (5) groups- Negative control (NC) group that was neither induced nor treated, Positive control (PC) that was induced but not treated, Low dose extract (LDE) group treated with 250mg/kg of the extract, High dose extract (HDE) group treated with 500mg/kg of the extract and Standard antihyperlipidaemic drug (SAD) group treated with 5mg/kg of Simvastatin. High fat diet was induced by feeding the rats with Ghee and Coconut oil in the ratio of 3:1 for a period of six (6) weeks, while administration of the treatments lasted for 4 weeks (3rd- 6th week), at the end of which animals were sacrificed and blood samples collected for subsequent serum lipid profile assays. Using standard analytical methods, Serum Total cholesterol (TC), Triglycerides (TG), High density lipoprotein cholesterol (HDL), Low density lipoprotein cholesterol (LDL-C), Very low density lipoprotein cholesterol (VLDL-C) analyses were performed, and Atherogenic coefficient (AC), Castelli's Risk Index I (CR I) and Castelli's Risk Index II(CR II) also calculated. Results showed that the mean concentrations (mg/dl) of the lipid profiles of the various groups ranges as follows: TC:80.53±7.14 (SAD) - 177.25±2.93(PC), TG:45.64±1.77 (SAD) - 159.74±4.69 (PC), HDL:35.19±3.81 (PC) - 57.81±4.31(SAD), LDL-C:18.09±2.79(NC) – 36.77±1.60 (PC), and VLDL-C:9.12±0.35(SAD) - 31.59 ± 0.73 (PC). For the other indices, Castelli's Risk Index I (CR I) ranges between 0.33±0.02 (SAD) - 1.05±0.11(PC), Castelli's Risk Index II(CR II): 1.40±0.08 (SAD) - 5.08±0.53 (PC), Atherogenic Coefficient (AC):0.39±0.08 (SAD) - 4.08±0.53 (PC), while that of Atherogenic Index of Plasma (AIP) was -0.10±0.02 (SAD) – 0.66±0.06. A closer look at the results revealed that in all instances, TC, TG, LDL-C, VLDL-C indices, CR I, CR II, AC, and AIP decreased significantly ($p<0.05$) in the LDE, HDE and SAD groups respectively when compared to the positive control group, while HDL increased significantly ($p<0.05$) relative to same PC group. This finding is further embellished by the percentage protection profile of the plant extracts, which is very high at both high and low doses. Percentage Protection conferred by the plant extracts were 61.76% (low dose) and 79.17% (high dose) respectively, both of which are reasonably favorably comparable to that (90.44%) of the standard antihyperlipidaemic antihypertensive drug. The efficacy of the extract in balancing lipid indices and atherogenic coefficient was as good as that of the standard antihyperlipidaemic antihypertensive drug (Simvastatin), since there was no significant difference ($P>0.05$) in the lipids reduction activities of the extract (particularly the higher dose) compared to that of the standard drug, thus suggesting its potential as antihyperlipidaemic antihypertensive agent. The results of this study have great implications for public health, as they suggest possible positive pharmacological role, therefore pharmacognostic value of *Diodia sarmentosa* in the search for solutions for hyperlipidaemic-mediated cardiovascular diseases, including hypertension.

Keywords: *Diodia sarmentosa*, Antihyperlipidaemic Activity, Antihypertensive, Cardiovascular Diseases, Public Health

C O N T E N T S

A R T I C L E I N F O

A U T H O R D E T A I L S
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1. Introduction

In today's world, the causes of serious ill-health are changing. Non-communicable diseases such as cardiovascular diseases are gradually replacing infection as a main cause of ill-health. Cardiovascular diseases (CVD) are among the major health problems that cause death worldwide. According to Sa'adah *et al*.[1], the prediction of the World health Organization (WHO) is that by 2030, 23.3 million people's deaths would be caused by cardiovascular diseases. Serious as this prediction may be, it is however of very serious epidemiological importance to note that, of the major causal factors responsible for cardiovascular diseases, hyperlipaemia is very crucial. Hyperlipidaemia is a condition caused as a result of steady consumption of high fat-rich diets. This is characterized by an elevation in serum lipids, including Total cholesterol (TC), Triglycerides (TG), Low density lipoprotein cholesterol (LDL-C), Very low lipoprotein cholesterol (VLDL-C) and reduction in High density lipoprotein cholesterol[1]. Hyperlipidaemia gives rise to the development of atherosclerosis, one of the major factors that bring about cardiovascular diseases such as hypertension, coronary heart disease and stroke, etc.[2].

Atherogenesis is a disorder that affects the artery wall by hardening of the artery walls (atherosclerosis), this is caused by the ingestion of low density lipoproteins, which leads to the buildup of cholesterol esters and formation of atherosclerotic plaques, thus giving rise to cardiovascular diseases [3]. Lipid Profile is a panel of blood tests that is carried out to evaluate the blood lipid composition, by determining the abnormalities in the lipids such as cholesterol and triglyceride, when they exceed their normal levels. These evaluations of lipid concentration and corresponding ratios are vital in the diagnosis and predictions of the development of cardiovascular diseases like atherosclerosis, coronary heart disease, hypertension, stroke etc.[3,4]. Elevations in total cholesterol, serum triglycerides, low-density lipoprotein-cholesterol and very low density lipoprotein-cholesterol concentrations above their normal levels are usually signs of dyslipidemia, and increased occurrence of heart related diseases [5]. Atherogenic indices and risk coefficient such as Castelli's risk index I (LDL-C/HDL), Castelli's risk index II (TC/HDL), Atherogenic coefficient (TC-HDL/HDL) and Atherogenic index of plasma (Log TG/HDL), have been shown to be better predictors of cardiovascular diseases than individual serum lipid parameters[3,5]. There are different ways of managing hyperlipidaemic condition like reduction of diet containing high lipids, use of lipid lowering drugs like statins and fibric acid derivatives, and also, natural materials from plants has been used as antihyperlipidaemic drugs [6].

The emergence of natural products from plants for the management of hyperlipidaemia came as a result of the high cost of the existing drugs, and side effects such as liver damage, gastric irritation, and nausea associated with the administration of the existing drugs[7]. The use of some plants and parts thereof in reducing high cholesterol in the body have been reported by some authors, as they were said to be affordable, and have little or no negative side effects in the body; while some examples of these plants include *Allium cepa, Cistanche tubulosa*, *Clitoria ternatea*, *Coriandrum sativum*, *Salvadora persica,* etc[8-11].

Diodia sarmentosa (Sw) commonly known as Zimbabwe flora or Tropical button weed is a straggling or procumbent perennial herb. It is from the family of rubiaceae with length of 1-4m long, often with many lateral branches from the main stem. Its Stems are up to 4m long, distinctly 4-angled and hairy on the angles. The leaves are opposite and up to 6.5cm long, its colour can be green to yellowish-green, with rough tubercle based hairs. It grows in evergreen forest particularly fringing 'mushitu' edges, open riverine vegetation, bush land and also on rocky places near rivers [12]. Local herbal folklore claims that this plant is efficacious in the local handling of certain health challenges, assertions most of which are scientifically yet to be verified. Meanwhile, the antiulcer potential of *Diodia sarmentosa* (whole plant)has been reported by Akah *et al.* [13], and the study of Umoh *et al.*[12] demonstrated the anti-inflammatory and analgesic activities of the plant, while Ijomone & Ekpe[14] reported on the anti-diabetic potential of same plant, In a recent study, Ezejiofor and Okoroafor[15] demonstrated sufficient biochemical and histopathological evidence of anticancer potentials of

Diodia Samentosa(SW) against diethyl nitrosamine-induced hepatocellular carcinoma (HCC) in Albino Rats. Presently however, there is no study regarding the antihyperlipidaemic and antihypertensive properties of this plant. Consequently, the present study aims to determine the antihyperlipidaemic potentials of ethanolic leaf extract of *Diodia sarmentosa* using animal models (Wistar rats) fed on high fat diet. Given the role of hyperlipidaemia in the aetiology and prognoses of some cardiovascular diseases such as atherosclerosis and hypertension, it is hoped that this study will be of great value in the search for natural, affordable and safe drugs against cardiovascular health challenges, particularly, those mediated through hyperlipidaemic route.

2. Materials and Methods

Plant Materials

Fresh samples of the plant were collected from the natural vegetation within the premises of the Federal University of Technology, Owerri (FUTO), and authenticated as *Diodia sarmentosa* at the Department of Crop Science of same institution. A portion of *Diodia sarmentosa* plant (as collected) is displayed hereunder (Plate A)

Plate A: Showing a sample of *Diodia sarmentosa* (Sw) plant leaves as collected from the vegetation

Chemicals and Reagents

Analytical grade chemicals and reagents were used for this study.

Experimental Animals

Males Wistar rats weighing between 150-200g were used for this study. The animals were purchased from Department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria.

Preparation of Plant Extract

Fresh leaves of *Diodia sarmentosa* (Sw) were air-dried at room temperature and then ground into fine powder using laboratory mortar and pestle. This leaves now in fine powder were soaked in 80% ethanol for a period of one week then filtered using Whitman filter paper to get the plant extract.

Experimental Site

The animals were acclimatized in the animal house of Biochemistry Department, Federal University of Technology, Owerri, under room temperature and relative humidity of 40-65% with a 12h natural light-dark cycle. The animals were granted free access to water and rats chew, and in all instances were handled in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) for animal experiment.

Grouping of animals and treatment

30 wistar rats (male) used for this experiment were grouped into five (5) groups, six (6) for each group;

NC Group: Animals received only standard pellet diet and water and considered as Negative Control

PC Group: Untreated High fat-diet induced rats considered as Positive control

LDE Group: High fat diet induced rats treated with 250mg/kg body weight of ethanolic leaf extract of *Diodia sarmentosa* (Sw)

Group HDE: High fat diet induced rats treated with 500mg/kg body weight of ethanolic leaf extract of *Diodia sarmentosa* (Sw). SAD Group: High fat diet induced rats treated with 5mg/kg weight of Simvastatin considered as Standard antihyperlipidaemic drug.

Preparation of Hyperlipidaemic Rats and Treatment Phase: Except the negative control group, the rats were placed on High-fat diet [Ghee and Coconut oil in the ratio of 3:1 for a period of six (6) weeks, as adapted from a previous study by Munshi *et al.* [16]. All animals were allowed uninhibited access to food and water, and body weight of rats was taken weekly. After the 3rd week, the blood samples were collected through ocular puncture and a confirmatory test was carried out to determine if the hyperlipidaemic induction was successful. From the 4th week to the end of the 6th week (i.e., 3weeks), the PC Group continued on the High fat diet while groups LDE, HDE and SAD continued with high fat diet and their respective treatments, while the NC Group remained on normal rat chew. The animals were orally administered the appropriate dosage of their respective treatments (*Diodia sarmentosa extracts* or SAD, as the case may be) once daily by intubation, using intravenous cannula tube. After the last treatment, the rats were allowed to fast for 24 hours, and then sacrificed (by anesthetizing them with chloroform vapour), and blood samples collected from each, through cardiac puncture. The obtained blood samples were centrifuged, separated to obtain serum (for analyses), and the later stored frozen in refrigerator until analyses.

Biochemical Analyses

Determination of Total Cholesterol Concentration

Determination of total cholesterol was done using the enzymatic (cholesterol esterase/oxidase/peroxidase) method of Allain *et al.* [17], as reported by Saidu *et al.* [18]

Determination of Triglyceride (TG) Concentration

For this, the glycerol phosphate oxidase/peroxidase method described by Bucolo& David [19] was used.

Determination of High Density Lipoprotein (HDL)-Cholesterol Concentration

The serum HDL-cholesterol concentration was measured using the phosphotungstate/Mg-cholesterol oxidase and peroxidase method described by Burstein *et al.* [20], as reported by Ojiako *et al*.[21].

Determination of Low Density Lipoprotein (LDL-C)-Cholesterol Concentration

Serum LDL-C level was determined according to the method of Assman *et al.* [22], as reported by Ojiako *et al*.,[21].

Determination of Very Low-Density Lipoprotein (VLDL-C)-Cholesterol Concentration

VLDL-Cholesterol was mathematically calculated using the formula described by Friedewald *et al.,*[23], as reported by Chikezie *et al*., [3]. In this formula, VLDL-C = Triglyceride/5

Atherogenic Indices and coefficient

The Atherogenic indices and coefficient were respectively calculated using the formula reported by Chikezie *et al*.,[3] as follows:

Castelli's Risk Index I (CRI-I) = LDL-C/HDL-C

Castelli's Risk Index II (CRI-II) = TC/HDL-C

Atherogenic Coefficient (AC) = (TC– HDL-C)/HDL-C

Atherogenic Index of Plasma (AIP) = log (TG/HDL-C)

$$\%\text{Protection of drug} = \frac{AC_{\text{positve control}} - AC_{\text{treatedgrp}} \times 100}{AC_{\text{positve control}}}$$

Where AC= Atherogenic Coefficient

Statistical Analysis

Data were analyzed using Computer Statistical Software for Social Sciences, (SPSS, version21).Descriptive statistics and one-way analysis of variance (ANOVA) were performed. Results for descriptive statistics were presented as mean ± Standard deviation of four determinations, while for ANOVA, the degree of statistical difference was accepted as significant at $p < 0.05$.

3. Results and Discussion

In Figure1, the results of the serum Total cholesterol levels of the various study groups are presented. The mean serum total cholesterol levels of the groups varied between 80.53mg/dl ± 7.14(lowest in the SAD group) and 177.25± 2.93 mg/dl (highest in the PC group).

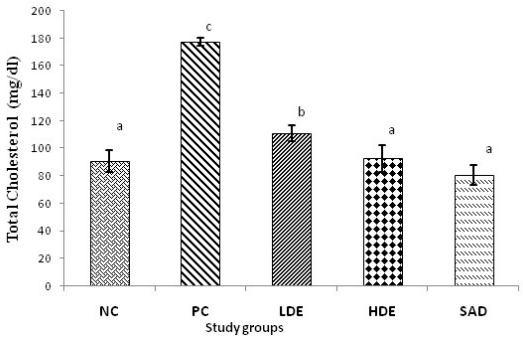

Figure 1: Result of Total Cholesterol (TC) levels in the studied groups.

Values are the means ± SD (n=4).Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different. Similarly, results of the serum Triglyceride levels of the various study groups are as shown in Figure 2. The mean triglyceride level of the groups varied between 45.64mg/dl ± 1.77 and 159.74mg/dl ± 4.69. While the PC Group showed the highest serum TG level, the SAD Group showed the lowest level of serum TG.

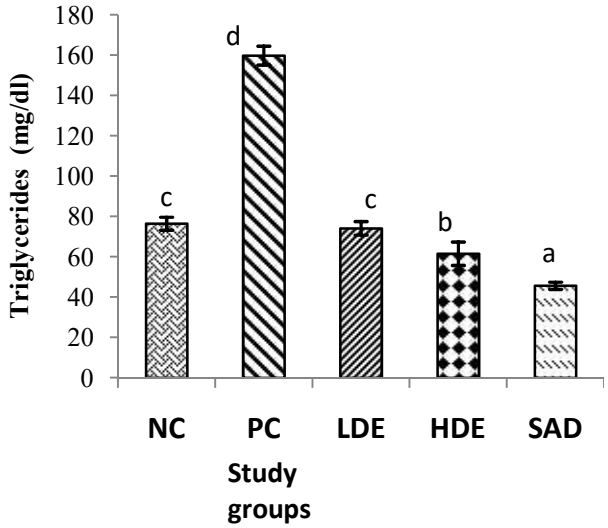

Figure 2: Result of Triglycerides (TG) levels in the studied groups.

Values are the means ± SD (n=4).Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different

In Fig. 3, the results of the serum High density lipoprotein cholesterol (HDL-C) levels of the various study groups are presented. The mean HDL-C levels of the groups varied between 35.18 ± 3.81 mg/dl and 57.80 ± 4.31mg/dl. The SAD Group showed the highest HDL-C level, while the PC Group showed the lowest serum HDL-C level.

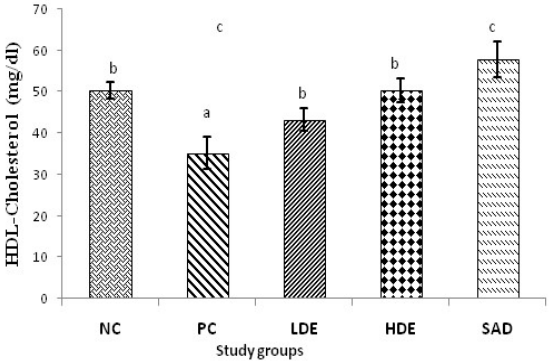

Figure 3: Result of High Density Lipoprotein Cholesterol (HDL-C) levels in the studied groups.

Values are the means ± SD (n=4). Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different.

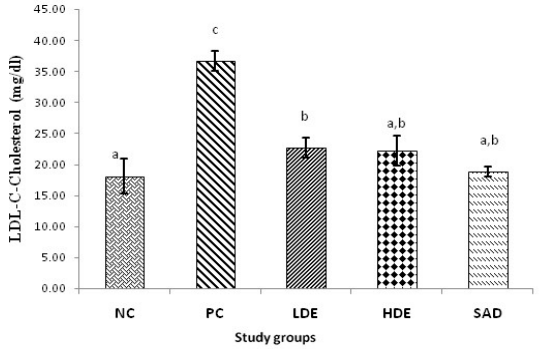

Figure 4: Result of Low Density Lipoprotein Cholesterol (LDL-C) levels in the studied groups.

Values are the means ± SD (n=4).Groups with different alphabets are significantly different (p<0.05), while groups with similar alphabets are not significantly different.

Figure 4. shows the result of the serum Low density lipoprotein cholesterol (LDL-C) levels of the various study groups. The serum LDL-C levels of the groups varied between 18.09± 2.79mg/dl and 36.77± 1.60 mg/dl. The PC Group showed the highest serum LDL-C level, while the NC Group showed the lowest serum LDL-C level.

The result of the serum Very low density lipoprotein cholesterol (VLDL-C) levels of the various study groups is as presented in Figure 5. The serum VLDL-C levels of the groups varied between 9.12± 0.35 mg/dl and 31.59± 0.73 mg/dl. The PC Group showed the highest serum VLDL-C level, while the SAD Group showed the lowest serum VLDL-C level.

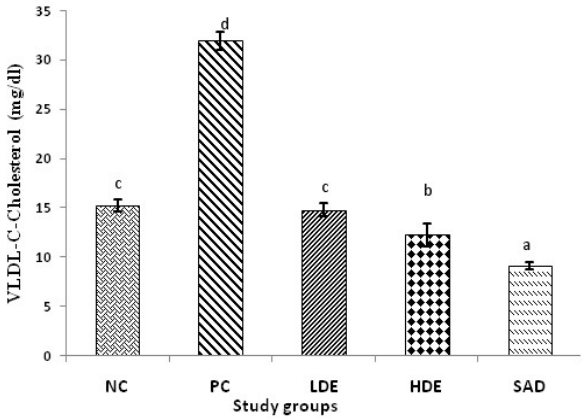

Figure 5: Result of serum Very Low Density Lipoprotein Cholesterol (VLDL-C) levels in the studied groups.

Values are the means ± SD (n=4). Groups with different alphabets are significantly different (p<0.05), while groups with similar alphabets are not significantly different.

In Figure 6, the results of Castelli's risk index I of the various study groups are presented. The CRI-I values of the groups varied between 0.33 ± 0.02 and 1.05 ± 0.11. Positive Control group showed the highest CRI-I value, while SAD Group showed the lowest value.

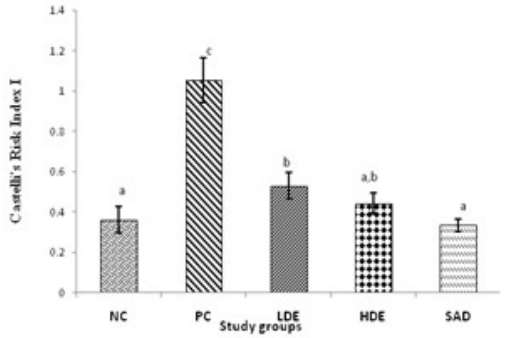

Figure 6: Result of Castelli's Risk Index I Calculations in the study groups.

Values are the means ± SD (n=4). Groups with different alphabets are significantly different (p<0.05), while groups with similar alphabets are not significantly different.

The results of Castelli's risk index II of the various study groups are as presented in Figure 7. The CRI-II values of the groups varied between 1.40 ± 0.08 and 5.08 ± 0.53. The PC Group showed the highest CRI-II value, while SAD Group showed the lowest CRI-II value.

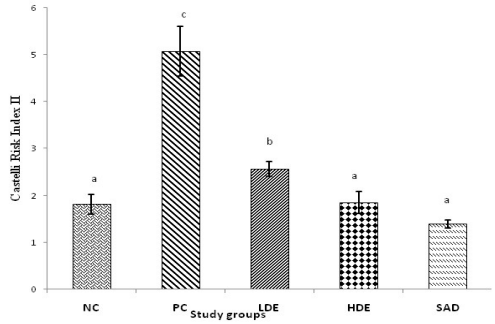

Figure 7: Results of Castelli's Risk Index II Calculations in the study groups.

Values are the means ± SD (n=4). Groups with different alphabets are significantly different (p<0.05), while groups with similar alphabets are not significantly different.

The results of the Atherogenic Coefficient (AC) calculations for the various study groups are as presented in Figure 8. The AC values of the groups varied between 0.39 ± 0.08 and 4.08 ± 0.53. The PC group showed the highest AC value, while the SAD group showed the lowest AC value.

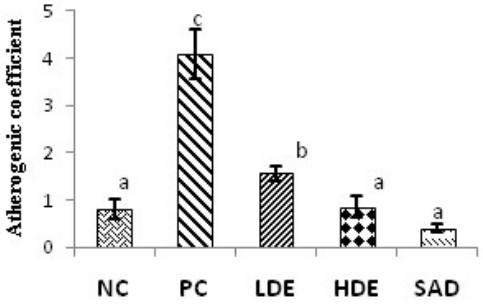

Figure 8: Result of Atherogenic Coefficient levels in the studied groups.

Values are the means ± SD (n=4). Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different.

The results of the Atherogenic index of plasma (AIP) calculations for the various study groups are as presented in Figure 9. The AIP values of the groups varied between -0.10 ± 0.02 and 0.66 ± 0.06. The PC group showed the highest AIP value, while the SAD group showed the lowest AIP value.

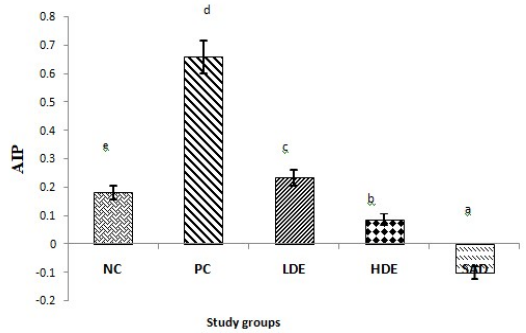

Figure 9: Result of Atherogenic Index of Plasma (AIP) in the studied groups.

Values are the means ± SD (n=4). Groups with different alphabets are significantly different ($p<0.05$), while groups with similar alphabets are not significantly different.

Table1 shows the percentage protection of the Low dose extract, High dose extract, and the standard antihyperlipidaemic drug (Simvastatin). The standard antihyperlipidaemic drug showed the highest percentage protection of 90.44%, followed by the High dose extract with a percentage protection of 79.17%, while the Low dose extract offered the least percentage protection of 61.76%.

Table 1: Percentage Protection of the different doses of the extract and that of the Standard antihyperlipidemic antihypertensive drug (Simvastatin).

Groups	% Protection
LDE	61.76
HDE	79.17
SAD	90.44

Discussion

Looking at the results, as furnished by the study, the mean serum concentrations (mg/dl) of the lipid profiles of the various groups shows that: Serum Total Cholesterol (TC)concentration ranges 80.53±7.14mg/dl, as recorded among the group receiving standard antihypertensive drug(SAD) to 177.25 ± 2.93mg/dl, as found among the non-treated positive control (PC) group, thus clearly revealing that, the least concentration of serum TC (80.53± 7.14mg/dl),was obtained among the group that received Simvastatin, a standard antilipidaemic antihypertensive drug (SAD). This efficacy in lipids reduction, with regards to total cholesterol, was closely followed by the high dose extract (500mg/dl) (TC=92.8150 ±9.85mg/dl), and then the low dose extract (250mg/dl) (TC=110.78±5.66mg/dl), since the groups that received these concentrations of the plant extracts as treatments respectively, showed dose dependent immediate lower total cholesterol levels in that order. For the high dose extract (HDE) group, the serum TC level showed no significant difference ($p>0.05$) when compared with those of both the SAD group and the Normal control(NC) group, thus revealing that the performance of the HDE is favorably comparable to that of SAD, and, that both SAD and HDE were able to reduce the serum TC to the range of levels found in the normal control group that never received any hyperlipidaemic induction, and therefore were normal ab-initio with respect to serum lipids. That both HDE, LDE, and SAD actually carried out lipids reduction activities were clearly evident, as there were significant differences ($P<0.05$) in the serum TC levels of the groups that received them as treatments respectively compared to the serum TC level in the positive control (PC) group in which, though induced with hyperlipidaemia, never received any treatment. Same pattern of result played out in almost all the lipid profile indices studied, except in the case of LDL-C(36.77±1.60 mg/dl - 18.09 ± 2.79 mg/dl),in which though the highest concentration, was still in the PC group, the lowest, this time, was in the NC group as against the usual SAD group. Apart from this variation, the rest of the lipid profiles also followed similar pattern of lipid reduction observed with respect to total cholesterol, because triglyceride (TG) ranged between 45.64±1.77 mg/dl(lowest in the standard antihypertensive drug (SAD) group and 159.74 ± 4.69 mg/dl(highest in the positive control (PC) group), VLDL-C ranged 9.12±0.35 mg/dl (lowest in SAD group) to 31.59 ± 0.73 mg/dl (highest in the PC group). For the other indices, Castelli's Risk Index I (CR I) ranged between 0.33±0.02(lowest in SAD group) and1.05 ± 0.11(highest in the PC group), Castelli's Risk Index II(CR II)ranged between 1.40±0.08(lowest in the SAD group) and 5.08±0.53(highest in the PC group), for Atherogenic Coefficient (AC), the range is 0.39±0.08(lowest in SAD group) and4.08±0.53(highest in the PC group), while that of Atherogenic Index of Plasma (AIP) remains between -0.10 ± 0.02 (lowest in the SAD group)and 0.66±0.06(highest in the PC group). For the serum HDL however, a total reversal of pattern was observed, because this time around, though the mean serum HDL levels of the groups varied between 35.18 ± 3.81 mg/dl and 57.80 ± 4.31mg/dl, the SAD group recorded the highest serum HDL level, while the PC group had the lowest serum HDL concentration (35.19 ± 3.81 mg/dl).

The very obvious lipids reduction potentials of *Diodia sarmentosa* plant extract is validated by the statistical relationships between the various parameters. A closer look at the results revealed that in all instances, Serum TC, TG, LDL-C, VLDL-C indices, CR I, CR II, AC, and AIP decreased significantly ($p<0.05$) in the low dose extracts (LDE), high dose extracts (HDE) and Standard antihyperlipidaemic drug (SAD)groups respectively when compared to the positive control (PC) group, while serum HDL increased significantly ($p<0.05$) relative to same PC group (Figs. 1-9). This finding is further embellished by the

percentage protection profile of the plant extracts, which is very high at both high and low doses. Percentage Protection conferred by the plant extracts were 61.76% (low dose) and 79.17% (high dose) respectively, both of which are reasonably favorably comparable to 90.44%obtained with the standard antihyperlipidaemic antihypertensive drug (Simvastatin) (Table 1).The efficacy of the extract in balancing lipid indices and atherogenic coefficient was as good as that of the standard antihyperlipidaemic antihypertensive drug (Simvastatin), since there was no significant difference($P>0.05$) in the lipids reduction activities of the extract (particularly the higher dose) compared to that of the standard drug, thus suggesting its potential as antihyperlipidaemic antihypertensive agent.

Discussing lipids and cardiovascular disease, Mayne [24] noted that there is a positive correlation between the risk of developing ischaemic heart disease and raised plasma total cholesterol and LDL cholesterol concentrations, and a negative correlation with plasma HDL cholesterol. According to this author, lowering high plasma LDL-C concentrations reduces the risk of cardiovascular disease. Hypercholesterolaemia is just one of the major risk factors of cardiovascular diseases; others include smoking and hypertension. Raised levels of HDL-cholesterol have protective effect against coronary heart disease, whereas diminished HDL-cholesterol, particularly in combination with elevated levels of triglyceride constitute an increased risk factors for cardiovascular diseases [25]. These scientific realities thus offer explanations for the presentation pattern observed here with respect to serum HDL-C, relative to other lipid profiles or indices, and also, further validating the lipid limiting/reduction properties of this plant extract, as suggested by the findings of this study, and consequently, the great possibilities it presents as a potential candidate for managing hyperlipidaemic-mediated cardiovascular disease, including arteriosclerosis and hypertension.

Various authors have reported decreases in High density lipoprotein cholesterol (HDL-C) and elevation of other lipid profiles like Total Cholesterol (TC), Triglycerides (TG), Low density lipoprotein cholesterol (LDL-C), Very Low density lipoprotein cholesterol (VLDL-C) and Atherogenic indices to give rise to conditions such as Hyperglycaemia, Hypercholesterolaemia, Hyperlipidaemia, Hypertension and sudden death, if proper management of these conditions are not taken [26-27]. Also, different plants like *Clitoria ternatea, Coriandrum sativum, Citrullus colocynthis, Aloe vera,* etc. have shown to possess remedies against various cardiovascular related diseases [10].

In this study, the PC group showed a significant decrease ($p<0.05$) in high density lipoprotein cholesterol (HDL-C), and a significant increase ($p<0.05$) in Total Cholesterol (TC), Triglycerides (TG), Low density lipoprotein cholesterol (LDL-C), Very Low density lipoprotein cholesterol (VLDL-C), Atherogenic coefficient and indices when compared to the negative control group (Figs. 1-9). This increase in the PC group was as a result of the high fat diet used for the study, which obviously caused an accumulation of high saturated fatty acid that possibly increased the levels of cholesterol and triglycerides in the rats. This also confirmed a successful induction of hyperlipidaemia in the experimental animals (treatment groups) before treatments commenced. This finding is in agreement with those of Neoman *et al*.,[28] and Olorunnisola *et al*. [29],which showed that different high fat diet formulations caused high elevations in serum TC, TG, LDL-C, VLDL-C levels, and a reduction in serum HDL-C concentration. Both doses of the extract, as also the standard drug, significantly ($p<0.05$) reduced the TC level of the hyperlipidaemic rats to levels found in normal control (NC) group, when compared to the positive control group (Fig 1). This shows that the extract possesses a cholesterol-lowering effect like the standard hyperlipidaemic drug. The extract at both doses also significantly ($p<0.05$) reduced elevated triglyceride levels when compared with the hyperlipidaemic positive control rats (Fig 2), thus reducing the occurrence of potential cardiovascular diseases like hardening of the arteries, and hypertriglyceridaemia that usually result from biosynthesis of high level of cholesterol. This means that this extract could be deployed in the management of hypertriglyceridaemia-induced atherogenesis. Similar Reduction in triglyceride level had been demonstrated by Olorunnisola *et al.,*[28] where *Tulbaghia violacea* was used to reduce high triglyceride level in obese rats.

High density lipoprotein cholesterol is 'good cholesterol' which carry cholesterol and lipids from the tissue to the liver for degradation. High density lipoprotein cholesterol helps to decrease the total level of cholesterol in the blood. Insufficient HDL-C leads to high level of cholesterol which leads to cardiovascular diseases. The higher the HDL-C, the lower the risk of atherosclerosis. The extract significantly increased ($p<0.05$) high density lipoprotein cholesterol level to levels found in normal control (NC) group, when compared to the decrease in the hyperlipidaemic positive control group (Fig 3). This reduced the total cholesterol concentration in the treatment groups. The effectiveness of *Diodia sarmentosa* in this regard is similar to that of *Medinilla speciosa* in increasing high density lipoprotein cholesterol [1].

Low density lipoprotein cholesterol (LDL-C) and Very low density lipoprotein cholesterol (VLDL-C) are lipid-rich lipoprotein cholesterol. Low density lipoprotein cholesterol is 'bad cholesterol' which transports cholesterol to the tissue and Very low density lipoprotein cholesterol transports triglycerides and fatty acid formed in the liver to the tissue. Low density lipoprotein cholesterol and Very low density lipoprotein cholesterol levels were significantly reduced ($p<0.05$) by the extract to levels found in normal control (NC) group, and the group receiving the standard drug (Simvastatin), when compared to the hyperlipidaemic positive control (PC) group (Fig. 4 and Fig. 5).

Atherogenic coefficient and indices (Castelli's risk index I, Castelli's risk II, Atherogenic coefficient and Atherogenic

index of plasma) have been reported to be better predictors of cardiovascular diseases than isolated lipid profiles [3]. The higher the atherogenic coefficient and indices, the higher the risk of atherosclerosis and coronary heart disease and vice versa. Our extract, at high and low doses, significantly ($p<0.05$) reduced all atherogenic coefficient and indices, when compared to the hyperlipidaemic positive control (PC) group (Figs. 6-9), a decrease that rivals the levels found in the negative control (NC) group. The efficacy of this plant extract with respect to reduction of atherosclerosis and cardiovascular diseases is similar to the findings of Chikezie *et al*.,[3] in which their herbal formulations of *Acanthus montanus, Asystasia gangetica, Gongronema latifolium* and *Solanum melongena* was used in reducing atherosclerosis in diabetic rats.

4. Conclusion

The overwhelming biochemical evidence furnished by this study suggests that crude Ethanolic leaves extract of *Diodia sarmentosa* (SW) possesses antihyperlipidaemic properties on High Fat Diet-Fed Wistar Rats. The plant is a potential cardiovascular plant, having demonstrated low atherogenic indices and ratios, and high percentage protection against hyperlipidaemia and by extension the potentially associated hyper lipid-mediated heart-related health challenges. Since this is the first time of reporting the antihyperlipidaemic properties of this plant extract and the consequent great possibilities it presents as a potential putative candidate for managing hyperlipidaemic-mediated cardiovascular diseases, including arteriosclerosis and hypertension, our report needs validation for pharmacological and pharmacognostic exploitations, because of the potential positive impacts of these on public health. It is hoped that results of our on-going studies would give clearer picture regarding the particular phytochemical constituent(s) of the plant that is responsible for the observed biochemical effects and the potential pharmacological implications.

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