Antihyperlipidaemic and anti-atherosclerotic activities of aqueous leaf extract of *L. africana* in streptozotocin induced diabetic rats

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Hyperlipidaemia is a usual and life threatening complication of diabetes mellitus. Consequently, a good anti-diabetic agent should reduce the progression of this complication. In view of this, the anti-hyperlipidaemic and anti-atherogenic activities of aqueous extract of *Lasianthera africana* was studied in streptozotocin induced diabetic rats. Extract treatment was administered at a dose of 1ml of 180 mg/ml of extract per kg body weight once daily for three weeks (21 days). Blood samples were collected thereafter by cardiac puncture for the analysis of lipid profile after the rats were exposed to chloroform vapour for 5 min. Results show that extract treatment caused significant (p<0.05) reduction in all lipid parameters analyzed when compared to the diabetic control. The reduction in total cholesterol was more for low density lipoprotein (LDL) (90%) than high density lipoprotein (HDL) (69%) by extract treatment when compared to the diabetic control by 94 and 75 percent respectively. The extract also caused significant (p < 0.05) reduction in cardiac risk ratio (CRR), atherogenic index of plasma (AIP) and the atherogenic coefficient respectively. The result therefore shows that the aqueous extract of *L. africana* possess anti-hyperlipidaemic and anti-atherosclerotic activities, a justification for its use by ethno-botanist in the treatment of diabetic complications.

Key words: Anti-hyperlipidaemia, anti-atherosclerotic, atherogenic coefficient, cardiac risk ratio, atherogenic index of plasma, *Lasianthera africana*.

INTRODUCTION

Dyslipidaemia is one common characteristic of diabetes mellitus resulting to the accompanied cardiovascular disorder often encountered by persons suffering from untreated diabetes. This complication often is the cause of lipid related disorders such as hyperlipilaemia, hypercholesterolaemia, stroke and cardiac arrest in such
persons. These are often life threatening and so may result to death if proper medical attention is not employed.

In low income earning economy, such as the ones noticeable in the sub-Saharan African regions, access to prompt medical attention may be delayed if not denied or completely absent, hence locals rely on herbal remedy which seems to be readily available from nature and cheaper to obtain (Mbagwu et al., 2011; McGrowder, 2013; Balde et al., 2006; Tanko et al., 2008). The treatment of diabetes and its complication with herbal remedy is a common historical practice among the local of the African continent and indeed the Niger Delta people of Nigeria (Obute et al., 2007). One of such herb in common use among the people is Lasianthera africana. Beside its use as herbs, it is also used as vegetables and food spices (Andi et al., 2008).

L. africana commonly known as “editang” by Efik speaking group and “gargbon” by the Ogonis of the Niger Delta is a plant that belongs to the order celestares and family Icacinacea. It comprises of 13 families of trees and shrub possessing simple leaves (Sofowora, 1993). It has been reported to possess cooling and purifying effects, as well as preventing internal bleeding (Adegolke et al., 2009). It is also reported to have bacteriostatic, fungicidal, anti-diabetic (Ekanem, 2006), antiplasmodial (Okokon et al., 2009), and antimicrobial (Nsor et al., 2012; Adegolke et al., 2008) activities. The phytochemical screening of the aqueous extract showed the active ingredients present to be more of alkaloids and carotenoids and trace amount of terpenoids, saponins, tannins, phytic and oxalic acids.

Most of the works reported the herbal activities of the extract in alcoholic, ethylacetate and butanolic media. We are however reporting its herbal potential in the aqueous medium, a form in which it is mostly consumed as food and vegetable spices, with the aim of ascertaining its effect on lipid metabolism in a diabetic induced experimental condition.

MATERIALS AND METHODS

Chemical

Streptozotocin used was obtained from Sigma-Aldrich, (USA) and the diagnostic kits for triglyceride, total cholesterol and high density lipoprotein were products of Randox Laboratories Ltd, Crumlin, England, UK. They were all of analytical grades. The reference drug (Daonil, a brand of glibenclamide) was purchased commercially from a local store.

Experimental animal handling

Healthy Wister albino rats (Rattus rattus) were purchased from the animal house of the Department of Biochemistry, University of Port Harcourt, Rivers State, Nigeria and transported in plastic cages to the Biological Laboratory of the Rivers State Polytechnic, Bori-Ogoni, Nigeria for housing. Fifty rats weighing between 180-200 g were kept in plastic cages covered with stainless wire gauge and given standard food pellets and water ad libitum to acclimatize. All the animals were maintained in normal conditions of light (12/24 h) and temperature (27±1°C). The acclimatization was for a period of one month before they were used for the experiment. The food pellets were that of grower mash, a product of Port Harcourt flourmills.

Extract preparation

Fresh leaves of L. africana was pulverized to coarse form of weight 100 g. The pulverized mass was dissolved in 1000 ml of distilled water and allowed to stand for 24 h on the laboratory bench with intermittent stirring to ensure proper extraction. The resulting solution was filtered with the aid of a muslin cloth and the filtrate concentrated to dryness by freeze drying for two weeks. The extract mass of 0.30 g obtained was dissolved in 100 ml of distilled water and used in the experiment as the aqueous stock solution from which the 180 mg/ml extract dose was obtained by appropriate dilution. This concentration of extract was obtained by a previous study as the amount capable of causing antihyperglycaemia in diabetic induced rats (Miikue-Yobe et al., 2015).

Induction of diabetes

Healthy rats were fasted for 18 h and then injected intraperitoneally with an aliquot (1.0 ml) of Streptozotocin (70 mg/kg body weight in 0.9% cold normal saline). The rats were observed for five days for signs of hyperglycaemia by daily monitoring of their blood glucose levels using the one touch glucometer (Prohp and Onogbe, 2009; Azadpakhta et al., 2010). The rats were allowed free access to water and food for the period. After the five days duration, rats with fasting blood levels ≥ 150 mg/dl (9.7 mmol/L) were considered diabetic. Twenty out of the fifty rats showed signs of hyperglycaemia. They were allowed an extra three days to stabilize before commencing treatment with extract and reference drug.

Estimation of plasma lipid profile

Assay of plasma triglyceride concentration

The plasma triglyceride concentration was enzymatically assayed using spectrophotometric method as reported by Ochei and Kolhatkar (2006). The determination was based on lipase/glycerol kinase/glycerol phosphate oxidase/peroxidase/chromogen reaction, a four step sequential reactions as represented below.

Test tubes labeled test, standard and blank contained 3.0 ml of the colour reagent to which 0.03 ml of serum obtained from the blood sample of experimental animals was added to the tubes labeled ‘test’ and same volume of the standard and distilled water added to the tubes labeled ‘standard’ and ‘blank’ respectively. The solutions were properly mixed and incubated at 37°C for 15 min. Absorbance readings of the test and standard solutions were taken at 420 nm wavelength while the blank was used to zero the machine.

Assay of plasma total cholesterol (TC) concentration

Plasma total cholesterol was assayed enzymatically with Commercial Test Kits (Randox Laboratories Ltd., Crumlin, England,
(UK).

Three test tubes were set up and labeled: T₁ (standard), T₂ (test sample) and T₃ (control sample). T₁ contained 0.01 ml of distilled water, T₂ contained 0.01 ml plasma while test tube T₃ contained distilled water. To each tube was added 1.0 ml of Randox cholesterol reagent. The contents were thoroughly mixed. Placed in water bath at 25°C for 10 min after which their absorbance (A) were read at 546 nm, against the blank, in a spectrophotometer.

**Assay of plasma HDL-cholesterol (HDL-C) concentration**

Plasma HDL-cholesterol was assayed enzymatically with commercial test kits (Randox Laboratories Ltd., Crumlin England, UK).

Three test tubes were set up and labeled; T₁ (blank), T₂ (Randox standard cholesterol) and T₃ (test sample). T₁ contained 0.01 ml of the supernatant from the tube above; T₂ contained 0.10 ml of the supernatant Randox from the standard cholesterol solution tube while T₃ contained 0.10 ml supernatant from sample tube. To each tube was added 1.0 ml of Randox cholesterol reagent. The contents were thoroughly mixed, placed in water bath at 25°C for 10 min after which their absorbance (A) were read at 546 nm, against the blank, in a spectrophotometer.

**Estimation of plasma VLDL, LDL-and non-HDL-cholesterol concentration**

Plasma VLDL-and LDL-cholesterol (LDL-C and VLDL-C) were calculated using the Friedelwald equation (Friedelwald et al., 1972) as shown in Equation “a” and “b”:

\[
\text{LDL-cholesterol (mg/dl)} = \frac{\text{total cholesterol- HDL-cholesterol-triglyceride/5}}{}
\]

(a)

\[
\text{VLDL} = \frac{\text{triglyceride/5}}{}
\]

(b)

The plasma non-HDL cholesterol concentration was determined as reported by Brunzell et al. (2008) as shown in equation “c”

\[
\text{Non-HDLcholesterol= [Total cholesterol] – [HDL-cholesterol]… (c)}
\]

**Evaluation of atherogenic indices**

The atherogenic indices were calculated as earlier reported by Ikewuchi and Ikewuchi (2011) using the formulas as shown in equations d-f:

\[
\text{Cardiac risk ratio (CRR)} = \frac{\text{[Total cholesterol]}}{\text{[HDL cholesterol]}} ...
\]

(d)

\[
\text{Atherogenic coeffient (AC) = } \frac{\text{[Total cholesterol]- [HDL cholesterol]}}{\text{[HDL cholesterol]}}
\]

All the concentrations in the calculation of the atherogenic indices were in mmol/L (Dobiasova, 2004).

\[
\text{Atherogenic Index of Plasma (AIP)} = \log \frac{\text{[Triglyceride]}}{\text{[HDL cholesterol]}}
\]

**Extract treatment of diabetic rats**

At the expiration of the period, the rats were weighed, fasted overnight and exposed to chloroform vapour for 5 min before blood samples for the lipid profile analysis was collected into plain bottles and centrifuged at 1000 g for 10 min and used for the analysis. Triglyceride, total cholesterol, high density lipoprotein (HDL) low density lipoprotein (LDL), non- high density lipoprotein (N-HDL) and very low density lipoprotein (VLDL) were obtained by calculation using the methods of Friedelwald et al. (1972) and Brunxel et al. (2008) while the atherogenic indexes were calculated according to the method of Ikewuchi and Ikewuchi (2011).

**Statistical analysis**

The data obtained were analyzed for statistical significance at the p<0.05 levels using the one way analysis of variance (ANOVA). The Duncan multiple treatment (Duncan et al., 1997) was used to further test the difference between treatment groups using IBM software SPSS13 package and the results were presented as mean with the standard error of the mean (Mean±SEM).

**RESULTS**

Table 1 shows the schedule of treatment analysis for the experimental rats (Azadbakha et al., 2010). The induced diabetic rats were injected intraperitoneally with 1.0 ml extract of 180 mg/ml per kg body weight of rats once daily for three weeks.

Table 2 shows the effect of aqueous extract of *L. africana* on lipid profile of streptozotocin induced diabetic rats.

Table 3 shows the atherogenic index of the effect of
aqueous leaf extract of *L. africana* on streptozotocin induced diabetic rats.

**DISCUSSION**

Hyperlipidemia is a known factor in coronary heart disease and atherosclerosis (Mengesha, 2006) while altered lipid metabolism and hyperlipidemia are commonly found as complications in diabetes mellitus. Results obtained in this study reflect the above trends showing the significant increase in the lipid profile of untreated experimental diabetic rats against the non-diabetic control and the diabetic treated rats respectively. Changes in the levels of HDL, LDL, TG and TC are predisposing indicators of Cardiovascular Disease (CVD).

The result as shown in Table 2 indicates that the extract treatment of the diabetic rats caused a significant reduction in all lipid profile parameters when compared to the diabetic untreated control. The result is significant as the reduction in total cholesterol is such that its total component is more of HDL than that of LDL which is a strong indicator of cardiovascular risk especially in the face of diabetes. Several reports have implicated HDL as a good antidote to cardiovascular disease, however consideration have not been given to its effect in the presence of increased total cholesterol fraction of HDL. Our result seem to suggest the likelihood that increased total cholesterol fraction of HDL as against that of LDL (Ogbera et al., 2009) as a positive indicator of CVD lowering effect by the extract. This is revealed in the result obtained by comparing the HDL level with that of the total cholesterol (the cardiac risk ratio).

The parameters so considered above are also atherogenic and their interpretation have received differing definitions from various groups and experts in the health care sector. This lack of consensus among these health care professionals is due to periodic changes and interrelationship between the risk factors which make it difficult to isolate the effect of a specific risk factor on lipid profile (Shivanand et al., 2012). The known fact as has been reported by some researchers is that among diabetics there appear to be a higher level of these lipids than in the normal non-diabetic condition while it increases higher in the absence of control (Shavanand et al., 2012). Our result as shown in Table 3 indicates that the aqueous extract of *L. africana* caused a significant reduction in cardiac risk ratio, a factor that is the ratio of the total cholesterol level to that of the level of HDL and is a determinant of the risk of onset of attack of cardiac arrest or myocardial infarction. It also reduced significantly the atherogenic Index of Plasma (AIP); a factor that tells the effect of treatment on the parameter of interest (El-Tantawy and Hassanin, 2007). It therefore provides the proof that the aqueous extract of the leaves of *L. africana* possesses antihyperlipidemic and anti-atherosclerotic potential in their effect on the diabetic induced rats as shown in this study.

**Table 2.** Effect of aqueous extract of *Lasianthera africana* on lipid profile in streptozotocin-induced diabetic rats.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Total Cholesterol</th>
<th>High density lipoprotein</th>
<th>Triglyceride</th>
<th>Low density lipoprotein</th>
<th>Very low density lipoprotein</th>
<th>Non-high density lipoprotein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>2.07 ± 0.15&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.34 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.52 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.62 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.14 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.72 ± 0.12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>1.57 ± 0.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.97 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.49 ± 0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.63 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.18 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.77 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glibenclamide treated</td>
<td>1.24 ± 0.32&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.53 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.45 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.65 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.66 ± 0.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.71 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Extract treated</td>
<td>1.44 ± 0.03&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.77 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.47 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.47 ± 0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.16 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.55 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results are Means ± SEM for n = 5 rats in each group. Experimental groups are compared with normal control and references drug, glibenclamide. Values with different superscripts in the same column are significantly different at the P < 0.05 level.

**Table 3.** Atherogenic indices of the effect of aqueous leaf extract of *L. africana* on streptozotocin-induced diabetic rats.

<table>
<thead>
<tr>
<th>Treatment Group</th>
<th>Cardiac risk ratio (CRR)</th>
<th>Atherogenic coefficient (AC)</th>
<th>Atherogenic index of plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>5.98 ± 1.21&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.99 ± 1.66&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.18 ± 0.00&lt;sup&gt;b,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Diabetic control</td>
<td>2.25 ± 0.58&lt;sup&gt;c,a&lt;/sup&gt;</td>
<td>1.25 ± 0.98&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.10 ± 0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glibenclamide treated</td>
<td>3.06 ± 3.25&lt;sup&gt;b,a&lt;/sup&gt;</td>
<td>2.06 ± 1.65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.22 ± 0.25&lt;sup&gt;a,c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Extract treated</td>
<td>1.89 ± 3.25d&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.88 ± 0.11d&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.10 ± 0.00&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results are Means ± SEM for n = 5 rats in each group. Experimental groups are compared with normal control and references drug, glibenclamide. Values with different superscripts in the same column are significantly different at the P < 0.05 level.
Conflict of Interests

The authors have not declared any conflict of interest.

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