The Hypoglycemic Effect of Aqueous Extract of the Anacardium occidentale Linn Leaves Grown in Nigeria on Normoglycemic Albino Rats

*, 1Saidu, A. N., 2Mann, A., 1Balogun, S.

1Department of Biochemistry, Federal University of Technology, Minna, Niger State, Nigeria
2Department of Chemistry, Federal University of Technology, Minna, Niger State, Nigeria

Corresponding Author: Saidu, A. N

Abstract

Extract of the Anacardium occidentale leaves is used in the traditional treatment of diabetic diseases. The hypoglycemic effect of leaves aqueous extract was investigated using normoglycemic albino rats. The leaves aqueous extract was found to contain phytochemicals such as alkaloids, saponins, flavonoids and tannins. The aqueous extract significantly lowered the blood glucose concentration in the normal rats (p>0.05). The extract produced about 33.7 % (305± 2.89 to 202 ± 4.04) mg/dl. The serum triglyceride decreases by 48.28 % (435± 4.50 to 225± 1.50) mg/dl in the experimental rats. While serum total protein decreases by 18.41% (27.7 ± 2.54 to 22.6 ± 0.32) g/d and weight increase was 4.75 % (134.6 ± 5.02 to 128.5 ± 1.15) g. The result obtained indicated that the extract has a moderate hypoglycemic effect and may be used locally for the treatment of diabetes mellitus.

Keywords: anacardium occidentale, diabetes mellitus, hypoglycemic effect, blood glucose level

INTRODUCTION

Diabetes mellitus is a major public health problem in the world. Diabetes mellitus is a metabolic disorder initially characterized by a loss of glucose homeostasis with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both (Barcelo and Rajpathak, 2001). Without enough insulin, the cells of the body cannot absorb sufficient glucose from the blood; hence blood glucose levels increase, which is termed as hyperglycemia. If the glucose level in the blood remains high over a long period of time, this can result in long-term damage to organs, such as the kidneys, liver, eyes, nerves, heart and blood vessels (Adetuyibi, 1976). Complications in some of these organs can lead to death (Pari and Saravanan, 2004). It is the fourth leading cause of death in most developed countries. Around four million deaths every year attributed to complication of diabetes. According to the World Health Organization estimate 3% of the world’s populations (194 million) have diabetes and is expected to double (6.3%) by the year 2030 (Wild et al., 2004), and much of this increase occurs in developing countries due to population growth, ageing, unhealthy diet, obesity and sedentary lifestyle (WHO, 2002) and in Nigeria, diabetes was found to be as high as 23.4 % among the high socio-economic group and 16% among the low socio-economic group (Nwafor and Owoji, 2001). Management of diabetes without side effect is still a challenge to the medical system. Currently available synthetic antidiabetic agents produce serious side effects such as hypoglycemic coma and hepatorenal disturbances (Gupta et al., 2008). Moreover, they are not save for use during pregnancy ((Rahman and Zaman, 1989). Hence, the search for safer and more effective hypoglycemic agents has continued. Ethnobotanical information indicates that plant species are used in the traditional management of diabetes (Abo et al., 2008; Li et al., 2004; Oubre et al., 1997; van Huyssteen et al., 2011).

Following the WHO’s recommendation for research on the beneficial uses of medicinal plants in the treatment of diabetes mellitus (WHO, 2002) and the increased demand by patients to use the natural product with antidiabetic activities (Tiwari and Rao, 2002), investigations on hypoglycemic agents has gained momentum. Several investigations have been conducted and many plants have shown hypoglycemic activity (Andrade-Cetto and Heinrich, 2005; Gbolade, 2009; Gidado et al., 2005; 2008; Grover et al., 2000; Olutimenyin et al., 2008; Momoh et al., 2011; Nagappa et al., 2003; Rahman and Zaman, 1989; Zibula and Ojewole, 2000). Though active principles have been isolated from some plants (Bekele, 2008; Ivorra et al., 1988; Latha et al., 2009; Ribnicky et al., 2006), some still remained to be identified. These reasons have necessitated the search for anew medication with proven and effective antidiabetic property. A. occidentale L. is one of the over 700 plants described to be beneficial in the treatment of diabetes mellitus (Day, 1995). Anacardium occidentale L. commonly known as
Cashew is found all over Nigeria. In the traditional Nigerian pharmacopoeia, stem-bark of *A. occidentale* is known for treatment of malaria fever, asthma, dysentery, toothache and sore gum (Mann *et al.*, 2003). The stem bark and leaves are also used to cure dermatitis and other skin diseases and pila (Izonzo, 1995). Similarly, extracts from roots, stems and fruits of *A. occidentale* have been used by the Cameroonian and other African countries’ traditional medicine (Sokeng *et al.*, 2001; Paris *et al.*, 1977). Indians also used the stem bark in herbal teas for asthma, cold, and congestion. Its fruit, juice and the nut oil are used in folk remedies for cancerous ulcers and elephantiasis. The seed oil is believed to be amobicidal used to treat gingivitis, malaria and syphilitic ulcers. Importantly, *A. occidentale* L. have been shown to display a wide spectrum of biological activities (Mota *et al.*, 1985; Ojewole, 2004), with experimental support for the empiric ethnopharmacological use of this plant in traditional medicine. The Nupe tribe of North Central, Nigeria uses the stem bark as cure for diabetes (Mann *et al.*, 2003). Studies of this plant from Cameroonian and other countries rather than Nigeria have shown to exhibit hypoglycaemic action (Kamtchouing et al., 1998; Sokeng *et al.*, 2001). However, there is no scientific evidence to support the hypoglycaemic effect of the leaves of *A. occidentale* grown in Nigeria. Therefore, the present objective of this study was to ascertain the scientific basis for the uses of this plant grown in Nigeria in the management of diabetes by evaluating the hypoglycaemic activity using aqueous leaf extract of the *A. occidentale* on normoglycemic albino rats.

**MATERIALS AND METHODS**

**Plant Material**

Fresh leaves of *Anacardium occidentale* were plucked within the Campus of Federal University of Technology, Minna, Niger State, Nigeria and it was identified and authenticated by a botanist in the Department of Biology, Federal University of Technology, Minna, Niger State, Nigeria. The leaves were air-dried under the shed at room temperature at Biochemistry laboratory, Department of Biochemistry, Federal University of Technology, Minna, Niger State, Nigeria. The dried plant material was manually powdered and the powder kept in polyethylene bags until used.

**Experimental Animals**

Six white albinos rats Wister strain weighing 100-291 kg of both sexes were purchased from Ahmadu Bello University, Zaria. Before and during the experiment, the rats were allowed free access to standard pellet diet and water. They were maintained in accordance with the recommendations (Guide for the Care and Use of Laboratory Animals, 1985). They were acclimatized for 3 weeks to adapt to laboratory environment. The rats were kept in standard cages with free access to food and clean water throughout the experimental period. All the animal experiments were conducted at Biochemistry laboratory, Department of Biochemistry, Federal University of Technology, Minna, Niger State, Nigeria (De Carvalho *et al.*, 2003).

**Preparation of Extracts**

200 g of powdered leaves of *A. occidentale* grown in Nigeria were exhaustively with (3 x 250 ml) hot distilled water (60 °C), mixed thoroughly and heated for 20-30 minutes in a water bath with continuous stirring. Hot aqueous extract was filtered under suction. All the extracts were combined and then concentrated on a water bath. The air-dried extract was then packed in a brown glass bottle with proper label and kept in a refrigerator until used for the experiment (Mann *et al.*, 2011).

**Preliminary Phytochemical Screening**

Standard screening test of the extract was carried out for various plant constituents. The crude extract was screened for the presence or absence of secondary metabolites such as alkaloids, steroidal compounds, phenolic compounds, flavonoids, saponins, tannins and anthraquinones using standard procedures (Harbone, 1998; Trease and Evans, 1989; Sofowora, 1993).

**Experimental Design**

The albino rats were divided into two groups of three each: Group 1 is the control while Group 2 is the experimental rats were given aqueous extract of *A. occidentale* grown in Nigeria. Group 1 rats were given pelletized standard diet and water only, while group 2 were given standard diet and free access to the extract suspended in distilled water as the only drinking source. After overnight fasting the blood samples were collected from the tail vein puncture after overnight (12 h) fasting. The serum was obtained via centrifugation, the blood was collected into clean dry centrifuge tube and was centrifuge for 10 minutes at 500 rpm. The serum was collected and transferred into tubes.

**Determination of Serum Total Protein (Biuret Reaction)**

In the Biuret reaction, a chelate is formed between Cu²⁺ ion and the peptide bonds of the protein in alkaline solution to form a violet coloured complex while absorbance is measured photometrically. The intensity of the colour produce is proportional to the concentration of protein in the sample.

\[
\text{Cu}^{2+} + \text{serum protein} \rightarrow \text{Copper- protein complex}
\]

1.0 ml of the reagent was pipette into a test tube and was added 0.02 ml of the reagent to 0.02 ml of the standard solution was added to a test tube. 1.0 ml blank was also prepared into a curvette the resulting mixture of sample and biuret was mixed thoroughly
and incubated for 10 minutes at 37 °C and the absorbance (A) of the sample and the standard at 540 mm against the blank reagent this was repeated for each animal in each group (Gornal et al., 1949).

**Calculation**

A sample x concentration of standard = total protein (g/dl)

A standard

A= Absorbance, C= Concentration

### **Determination of serum triglycerides (Enzymatic-colorimetric method)**

The method is based on the enzymatic hydrolysis of serum triglycerides and free fatty acids (FFA) by lipoprotein lipase (LPL). The glycerol is phosphorylated by adenosine triphosphate (ATP) in the presence of glycerol kinase (GK) to form glycerol-3-phosphate and adenosine diphosphate. Glycerol 3-phosphate is oxidised by glycerol phosphate oxidases to form dihydroxyl acetone phosphate (DHAP) and hydrogen peroxide. A red chromogen is produced by peroxide (POD) catalysed coupling of 4-amino antiphyrine (4-AA) and phenol with hydrogen peroxide (H₂O₂), proportional to the concentration of triglyceride in the sample (Barham and Trinder, 1972).

\[
\text{Glycerol} + 3\text{H}_2\text{O} \xrightarrow{\text{LPL}} \text{Glycerol} + 3\text{FFA}
\]

\[
\text{Glycerol} + \text{ATP} \xrightarrow{\text{GK}} \text{Glycerol-3-P + ADP}
\]

\[
\text{Glycerol-3-P} + \text{O}_2 \xrightarrow{\text{GPO}} \text{DHAP} + \text{H}_2\text{O}_2
\]

\[
\text{4-AA} + 4\text{phenol} \xrightarrow{\text{POD}} \text{Quinonamine} + \text{H}_2\text{O}
\]

The reagent and sample were used at room temperature, 1.0 ml of mono reagent was measured into a test tube as blank; 1.0 ml of the mono reagent was pipette into a test tube containing 0.01 ml of the standard. Both were mixed thoroughly as the standard. 1.0 ml of mono reagent was pipette into a test tube containing 0.01 ml of the serum sample, it was mixed thoroughly and all the test tubes were incubated for 5 minutes at 37 °C. Each of the absorbance of the sample and the standard at 500 mm against the blank reagent were determined.

### **Body Weight Determination**

Each animal in the group were eight weekly with an electro balance.

### **RESULTS**

Table 1 shows the result of the phytochemical screening while table 2, 3, 4 and 5 shows of the result of the effect of the aqueous extract of *A. occidentale* grown in Nigeria on glucose, serum protein, triglyceride and weight.

### **Table 1: Phytochemical screening result of the plant extract**

<table>
<thead>
<tr>
<th>Group</th>
<th>Test</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Dragendorff’s reagent</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Wagner’s</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>Lead acetate NaOH</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>Frothing test</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric chloride</td>
<td>+</td>
</tr>
<tr>
<td>Balsams</td>
<td>Balsam</td>
<td>-</td>
</tr>
<tr>
<td>Resins</td>
<td>Copper acetate</td>
<td>-</td>
</tr>
</tbody>
</table>

Key: + = positive; - = negative

### **Table 2: Effect of aqueous extract of *A. occidentale* grown in Nigeria on blood glucose concentration (mg/dl) in normoglycemic rats**

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Control (Normal)</th>
<th>Treated (Normal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>00</td>
<td>222 ± 4.62</td>
<td>305 ± 2.89</td>
</tr>
<tr>
<td>01</td>
<td>223 ± 2.83</td>
<td>270 ± 4.40</td>
</tr>
<tr>
<td>02</td>
<td>218 ± 277</td>
<td>256 ± 1.15</td>
</tr>
<tr>
<td>03</td>
<td>229 ± 2.02</td>
<td>227 ± 2.08</td>
</tr>
<tr>
<td>04</td>
<td>221 ± 1.96</td>
<td>2024 ± 4.04</td>
</tr>
</tbody>
</table>

Values are means ± S.E. M of 3 rats.

### **Table 3: Effect of aqueous extract of *A. occidentale* grown in Nigeria on serum triglyceride (mg/dl) in normoglycemic rats**

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Control (Normal)</th>
<th>Treated (Normal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>00</td>
<td>281 ± 0.57</td>
<td>435 ± 4.50</td>
</tr>
<tr>
<td>01</td>
<td>279 ± 5.43</td>
<td>418 ± 3.98</td>
</tr>
<tr>
<td>02</td>
<td>277 ± 5.02</td>
<td>378 ± 1.15</td>
</tr>
<tr>
<td>03</td>
<td>263 ± 7.13</td>
<td>222 ± 196</td>
</tr>
<tr>
<td>04</td>
<td>268 ± 1.73</td>
<td>225 ± 1.50</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M of 3 rats.

### **Table 4: Effect of aqueous extract of *A. occidentale* grown in Nigeria on serum total protein (mg/dl) in normoglycemic rats**

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Control (Normal)</th>
<th>Treated (Normal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>00</td>
<td>21.1 ± 2.00</td>
<td>27.70 ± 2.54</td>
</tr>
<tr>
<td>01</td>
<td>20.0 ± 1.15</td>
<td>18.48 ± 1.62</td>
</tr>
<tr>
<td>02</td>
<td>19.7 ± 0.75</td>
<td>20.38 ± 3.52</td>
</tr>
<tr>
<td>03</td>
<td>20.5 ± 1.62</td>
<td>18.10 ± 1.73</td>
</tr>
<tr>
<td>04</td>
<td>20.6 ± 1.33</td>
<td>22.50 ± 0.32</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M of 3 rats.

### **Table 5: Effect of aqueous extract of *A. occidentale* grown in Nigeria on weight (g) of normoglycemic rats**

<table>
<thead>
<tr>
<th>Weeks</th>
<th>Control (Normal)</th>
<th>Treated (Normal)</th>
</tr>
</thead>
<tbody>
<tr>
<td>00</td>
<td>100.3 ± 2.54</td>
<td>128.5 ± 1.15</td>
</tr>
<tr>
<td>01</td>
<td>110.5 ± 3.58</td>
<td>130.5 ± 3.52</td>
</tr>
<tr>
<td>02</td>
<td>105.6 ± 4.04</td>
<td>132.0 ± 17.3</td>
</tr>
<tr>
<td>03</td>
<td>103.7 ± 5.14</td>
<td>131.9 ± 1.96</td>
</tr>
<tr>
<td>04</td>
<td>107.2 ± 0.12</td>
<td>134.6 ± 5.02</td>
</tr>
</tbody>
</table>

Values are means ± S.E.M of 3 rats

### **DISCUSSIONS**

Medicinal values of plants are very useful for mankind and have no side effect on human health. A variety of medicinal plants are used by Nigerians for the management of diabetes mellitus. One of such plants is the leaves of *A. occidentale* grown in
Nigeria. Plants possess a large number of chemical constituents which are responsible for their medicinal properties. In the present investigation, aqueous extract of the leaves of *A. occidentale* grown in Nigeria were screened for phytochemicals commonly found in hypoglycemic plants. Phytochemical screening was done using colour forming and precipitating chemical reagents on the dried leaves of *A. occidentale* grown in Nigeria to generate preliminary data on the constituents of the plant extracts.

The results obtained from the tests were summarized in Table 1. The chemical test results indicated the presence of alkaloids, flavonoids, tannin and saponins in the extract of the leaves of *A. occidentale* grown in Nigeria which compared favorable with the earlier studies (Kametchouing et al., 1998; Sokeng et al., 2001). Natural products play a very important role in our daily life. Plants with hypoglycemic and antihyperglycemic activities may contain one or more chemical constituents (Gupta et al., 2008). Classes of chemical compounds isolated from plants are documented to have the potential to decrease the blood glucose level (Ragavan and Krishnakumari, 2006; Miura et al., 2005; Oubre et al., 1997). Thus, the significant antidiabetic effect of extracts of *A. occidentale* could be due to the possible presence of the aforementioned constituents in the part of the plant used in this particular study, which could act synergistically or independently enhancing the activity of glycolytic and glycogenic enzymes.

The present results of aqueous leaves extract of *A. occidentale* grown in Nigeria examined for its effect on blood glucose similar to the report of Tanko et al. (2000), while serum total protein, serum triglyceride and the body weight in normoglycemic rats displayed some remarkable differences. The Table 1 shows that the plant *A. occidentale* contains chemical compounds like alkaloids, saponins, flavonoids and tannins which in agreement with previous report (Dietewa, 2004). Based on the increasing number of reports on blood glucose reduction in association with some saponins (Dietewa, 2004) and alkaloids (Boikent et al., 2000) isolated from other medicinal plants, it is likely that the active principles could be present in one or more of the chemical substances. These compounds stimulate influx of Na\(^{+}\) and Ca\(^{2+}\) ions by inhibiting the Na\(^{+}\)-Ca\(^{2+}\) exchanger thereby stimulating the influx of Ca\(^{2+}\) into the inner membrane, hence there is membrane depolarization. This activates the synthesis of ATP through oxidative phosphorylation. This mechanism feed forward glucose, stimulated insulin secretion. The ATP produced mediates the secretion of insulin by insulin secretagogues. Table 2 to table 5 shows that *A. occidentale* cultivated in Nigeria produce hypoglycaemic and other effect in normoglycemic rats. This observation is in line with previous reports on the leaves (Sokeng et al., 2001; Tédong et al., 2006). A closer look revealed significant hypoglycaemic effect of the aqueous extract since it lowered the blood glucose to nearly half the normal value. From the table 2, the aqueous leaves of *A. occidentale* cultivated in Nigeria produces about 33.7% decreases in the blood glucose level (305±2.89 to 202±4.04) mg/dl at the end of the fifth week. The extract significantly lowered the basal blood glucose concentration in the normoglycemic rat at (P<0.05) (Sanchez et al., 2000). From table 3, there was a decrease in the total serum triglyceride concentration in both the normoglycemic treated rats where as the concentration of the normoglycemic control rats fluctuated. However, by second week, there was a decrease in the triglyceride concentration which continued to decrease weekly reflecting the effect of the aqueous leaves extract.

Furthermore, due to the fact that triglyceride is the most preferred source of energy when glucose is depleted, it is mobilized back to the stored depot (adipose tissue); thereby accounting for the decrease in triglyceride concentration in the blood as well as significant difference between the blood glucose level and serum triglyceride concentration. From table 4, there was a decrease in the total serum concentration in the normoglycemic treated rats by approximately 18.4 % (27.41 ± 2.54 to 22.6 ± 0.32) g/dl whereas for the normoglycemic control rats, their total serum protein concentration fluctuated. However by week two, there was a decrease in total serum protein this is similar to the triglycerides because protein which was also being mobilized and used for energy are at this stage demobilized back to the store due to the effect of the aqueous leaves extract, as glucose is used for energy. Table 5, shows that there was an increase in weight which may be due to the mobilization of fats back to the adipose tissue, hence protein synthesis and lipogenesis. In conclusion the result obtained indicates that the aqueous extract of the *A. occidentale* grown in Nigeria revealed the presences of alkaloids, flavonoids, tannins and saponins. Compounds belonging to these chemical groups are known to be bioactive for the management of diabetes. A significant hypoglycaemic activity for the leaves extract of *A. occidentale* grown in Nigeria was observed. Hence, the data obtained from these experiments have provided the chemical basis for the wide use of this plant as therapeutic agent for managing diabetes and may serve as a good source for search for antidiabetic drugs. Furthermore studies are required to fractionate, purify and identify the active principle(s) present and to probe into biochemistry of the leaves of *A. occidentale* cultivated in Nigeria.
REFERENCES


