The Study of the effects of n-butanol fraction of *Alchornea cordifolia* leaf extract on lipid profile and liver enzymes in streptozotocin-induced diabetic rats

Mohammed, R.K., Ibrahim, S., Atawodi, S.E., Eze, E.D., Suleiman, J.B. and Malgwi, I.S.

*Department of Biochemistry, Faculty of Science Ahmadu Bello University, Zaria, Nigeria.*

**ABSTRACT**

*Alchornea cordifolia* (Schumach. & Thonn.) Müll. Arg. (Euphorbiaceae) is a widely distributed plant in Africa especially in Nigeria. The study investigated the effects of n-butanol fraction of *Alchornea cordifolia* leaf extract on lipid profile and liver enzymes in streptozotocin-induced diabetic rats. To accomplish this, the animals were made diabetic by single intraperitoneally injection of 60 mg/kg body weight dose of streptozotocin dissolved in 0.1 ml fresh cold citrate buffer pH 4.5 into 16 h-fasted rats. After which the diabetic animals were randomly divided into the following groups: Group I served as the normal control, Group II served as diabetic control, while Group III to Group VI were treated with 200, 400 and 800 mg/kg b w of the plant extract fraction and glibenclamide 10mg/kg b w respectively by oral gavage for a period of 4 weeks. At the end of treatment period all animals from each group were euthanized and blood samples collected by cardiac puncture. There was a statistically significant (p< 0.05) reduction in blood glucose level in the group treated with 200mg/kg b w with a higher decrease (p < 0.01) observed with 400 and 800mg/kg b w. However, after day 14, 21 and 28, the blood glucose level was highly significantly (p < 0.01) reduced in all extract treated groups when compared to the diabetic control group. The study also revealed a significantly decreased (p<0.05) serum total cholesterol, triglyceride and low-density lipoprotein and significantly elevated (p<0.05) serum levels especially in the groups treated with 400 and 800 mg/kg b w of the plant extract when compared to the diabetic control group. There was also a significantly reduced (p<0.05) serum liver enzymes, AST, ALT and ALP as well as total and direct conjugated bilirubin levels in all groups that received various doses of the plant extract when compared to the diabetic control group. In can be concluded that the plant possess antidiabetic property suggesting that the plant may be useful in the management of dyslipidemia, a secondary complication that usually occur in diabetic condition.

**Key words:** *Alchornea cordifolia*, alanine aminotransaminase, alkaline phosphatase, aspartate aminotransaminase, cholesterol, diabetes mellitus, low density lipoprotein, streptozotocin, triglyceride,

**Introduction**

Diabetes mellitus is a chronic metabolic disorder with numerous complications. It is characterized by chronic high blood glucose levels leading to increased morbidity and mortality. Diabetes if not controlled results in structural and functional changes in various target tissues and organs (Fauci et al., 2008). Dyslipidemia is the major risk factors for macrovascular complications leading to cardiovascular disease (CVD) in type 2 diabetes mellitus. In addition to this, endothelial dysfunction, platelet hyperactivity, impaired fibrinolytic balance and abnormal blood flow may accelerate atherosclerosis and increased risk of thrombotic vascular events (Colwell and Nesto, 2003). Macrovascular disease is the most common cause of morbidity and mortality especially in type 2 diabetes mellitus (Koskinen, 1998). Macrovascular disease is defined as illnesses affecting the larger arteries supplying the heart, brain, and the legs, thereby causing ischemic heart disease, cerebrovascular disease, and peripheral vascular disease. In patients with diabetes, alteration in distribution of lipid increased risk of atherosclerosis. Specifically, insulin resistance and insulin deficiency was identified as phenotype of dyslipidemia in diabetes mellitus (Krauss and Siri, 2004; Chahil and Ginsberg, 2006). And this is usually characterized with high plasma triglyceride, total cholesterol, LDL-cholesterol level and low HDL-cholesterol level (Moordian, 2009). Hepatobiliary disorders, such as inflammation, necrosis or fibrosis of non-alcoholic fatty liver disease, cirrhosis, hepatocellular carcinoma, hepatitis C, acute liver failure, and cholelithiasis are complications of diabetes (Balazs and Halmos, 1995; Tolman et al., 2004). Therefore combating diabetes mellitus goes far beyond mere glycaemic control. Streptozotocin is frequently used to induce experimental type 1 diabetes (Yamagishi et al., 2001). The cytotoxic action of STZ is mediated by free radicals and STZ has toxic and carcinogenic effects on the pancreas, liver and kidneys (Okawa and Doi, 1983). Currently available therapies for diabetes such as insulin, sulfonylureas, biguanides, α-glucosidase inhibitors, and glinides, suffer from various adverse effects, thus, managing diabetes without any side effects is still a challenge to the workers.
(Saxena and Kishore, 2004), and hence the search for more effective and safer therapeutic agents in eradicating diabetic syndromes has continued to be an important area of investigation. To cope with severe problems associated with using of synthetic anti-diabetic drugs, there is a need to look for more efficacious drugs with lesser side effects and also of low cost (Goutam, 2011). *Alchornea cordifolia* is widespread in secondary forest and riverine forest, especially in marshy areas but sometimes in drier sites, from sea-level up to 1500 m altitude. It is commonly used as a medicinal plant throughout its area of distribution. The leaves are mostly used, but also the stem bark, stem pith, leafy stems, root bark, roots and fruits enter in local medicine (Adewunmi *et al.*, 2001; Ebi, 2001). In Nigeria, the leaves or leafy stems, as an infusion or chewed fresh, are taken for their sedative and antispasmodic activities to treat a variety of respiratory problems including sore throat, cough and bronchitis, genital-urinary problems including venereal diseases and female sterility, and intestinal problems including gastric ulcers, diarrhoea, amoebic dysentery and worms (Banzouzi *et al.*, 2002). They are also taken as a blood purifier, as a tonic and to treat anaemia and epilepsy. A decoction of bruised fruit is taken to prevent miscarriage. The sap of the fruit is applied to cure eye problems and skin diseases (Obadoni and Ochuko, 2002; Osadebe, and Okoye, 2003). However, its use in the management of diabetes mellitus and its associated complication has not been fully elucidated. Therefore, the present study is aimed at evaluating the effect of *Alchornea cordifolia* on lipid profile and liver enzymes in streptozotocin-induced diabetic wistar rats.

**Materials and Methods**

**Chemicals and drugs used:**

Streptozocin (STZ) was purchased from Sigma chemicals (St Louis U.S.A). Glibenclamide was purchased from pharmaceutical store in Zaria, Kaduna state, while Accu-check Advantage a digital glucometer was used for the determination of blood glucose levels.

**Plant material used:**

Fresh leaves of *Alchornea cordifolia* were collected from old Karu village, Karu Local government of Nasarawa State, Nigeria in the month January 2011. The plant was then taken to the herbarium unit of Biological Science Department Ahmadu Bello University, Zaria, Kaduna state, where the plant was identified by Mal. M. Musa and a voucher specimen (Number 401) deposited.

**Plant extract preparation:**

The fresh leaves *Alchornea cordifolia* collected were air dried under the shade and ground into fine powder. The powder (400g) was macerated in 2.0 L of distilled water at room temperature for 24 h. It was then filtered using a filter paper (Whatmann size 1). The filtrate was then partitioned with n-Butanol to get an n-Butanol fraction which was then evaporated to dryness in an oven at 30°C. A brownish residue weighing 8g was obtained and kept in a sealed container refrigerated until it was reconstituted.

**Acute toxicity study:**

The lethal doses (LD<sub>50</sub>) of fresh leaves of *Alchornea cordifolia* was carried out by method of Lorke (1983).

**Phytochemical screening:**

The methods of analysis employed were those described by Beach and Turner (1975).

**Animals:**

Strains of albino wistar rats of both sexes that weighed between 150 – 200 g were obtained from the Department of Human Physiology, Animal House, Ahmadu Bello University, Zaria. The animals were kept and maintained under laboratory condition of temperature, humidity and light. The animals allowed to acclimatized for two weeks, but were allowed free access to water, before commencement of the experiments.

**Induction of experimental diabetes mellitus:**

Diabetes mellitus was induced by single intraperitoneal injection of 60 mg/kg body weight dose of streptozotocin dissolved in 0.1 ml fresh cold citrate buffer pH 4.5 into 16 h-fasted rats. Three days after Streptozotocin injection, blood was taken from tail artery of the rats [14]. Rats having blood glucose levels
greater than 200mg/dl were considered diabetic and included in the study. The diabetic rats were then divided randomly into different groups before the commencement of treatment.

Experimental design:

In the experiment, a total of 36 rats were used, the animals were divided into 6 groups of 6 rats each as follows:

- Group 1: Normal control and received 1ml of distilled water
- Groups 2: Diabetic control and treated with 1ml of distilled water
- Group 3: Diabetic and received 200mg/kg b w n-butanol fraction of A. cordifolia leaf extract
- Group 4: Diabetic and received 400mg/kg b w n-butanol fraction of A. cordifolia leaf extract
- Group 5: Diabetic and received 800mg/kg b w n-butanol fraction of A. cordifolia leaf extract
- Group 6: Diabetic and received Glibenclamide 10mg/kg b w

All regimen was given by gavage orally for a duration spanning for 28 days.

Determination of blood glucose levels:

All blood samples were collected from the tail artery of the rats after every seven days. Blood glucose levels was determined by the glucose-oxidase principle [14] using a digital glucometer (Accu-chek Advantage) and expressed in the unit of mg/dl.

Determination of lipid profile:

These were determined spectrophotometrically, using enzymatic colometric assay kits (Randox Laboratories Limited kits, Unite kingdom) as follows:

Determination of serum total cholesterol:

The serum level of total cholesterol was quantified after enzymatic hydrolysis and oxidation of the sample as described by method of Stein (1987). 1000µl of the reagent was added to each of the sample and standard. This was incubated for 10 minutes at 20-25 °C after mixing and the absorbance of the sample (A sample) and standard (A standard) was measured against the reagent blank within 30 minutes at 546nm.

Determination of serum triglyceride:

The serum triglyceride level was determined after enzymatic hydrolysis of the sample with lipases as described by method of Tietz (1990). 1000µl of the reagent was added to each of the sample and standard. This was incubated for 10 minutes at 20-25 °C after mixing and the absorbance of the sample (A sample) and standard (A standard) was measured against the reagent blank within 30 minutes at 546nm.

Determination of serum high-density lipoprotein cholesterol:

The serum level of HDL-C was measured by the method of Wacnic and Albers (1978). Low-density lipoproteins (LDL and VLDL) and chylomicron fractions in the sample were precipitated quantitatively by addition of phosphotungstic acid in the presence of magnesium ions. The mixture was allowed to stand for 10 minutes at room temperature and centrifuged for 10 minutes at 4000 rpm. The supernatant represented the HDL-C fraction. The cholesterol concentration in the HDL fraction, which remained in the supernatant, was determined.

Determination of serum low-density lipoprotein cholesterol:

The serum level of (LDL-C) was measured according to protocol of Friedewald et al., (1972) using the relationship below:

\[ \text{LDL-C} = \frac{\text{TC-TGL}}{5} + \text{HDL-C} \]

Determination of serum liver enzymes:

The serum enzymes Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) were determined spectrophotometrically, using enzymatic colometric assay kits according to the laboratory procedures of Randox Laboratories Limited kits, United kingdom.
Statistical analysis:

All data obtained were expressed as mean ± SEM. The data were statistically analyzed using one-way analysis of variance (ANOVA) with Tukey’s multiple comparison post hoc tests to compare the level of significance between control and experimental groups. All statistical analysis was done using SPSS version 17.0 software. The values of p < 0.05, p < 0.01 and p < 0.001 were considered as significant.

Results:

Acute toxicity study:

In the present study, treatment of the animals with various doses of n-butanol fraction of *Alchornea cordifolia* leaf extract showed no signs of toxicity and no deaths were recorded. Therefore, the LD₅₀ of n-butanol fraction of leaf extract *Alchornea cordifolia* was safe at least up to the dose of 5000mg/kg b w.

Preliminary phytochemical screening:

Preliminary phytochemical screening of the plant extract revealed the presence of cyanogenetic glycosides, saponins, carbohydrate, flavonoids, tannins, cardiac glycosides and steroids and Triterpenoids.

Effect of n-butanol fraction of *Alchornea cordifolia* on blood glucose level in streptozotocin-induced diabetic Wistar rats:

Table 1 shows the mean blood glucose level of control and the experimental groups. The study revealed that the blood glucose levels did not differ significantly (p >0.05) within and between groups after day 1 and when compared to the diabetic control group. But there was a statistically significant (p< 0.05) decrease in the blood glucose level in the group treated with 200mg/kg b w with a higher decrease (p < 0.01) observed with 400 and 800mg/kg b w. However, glibenclamide (10mg/kg b w) gave a better effect with (p<0.001) after day 7 when compared to the control group. However, after day 14, 21 and 28, the blood glucose level was highly significantly (p < 0.01) reduced in all extract and glibenclamide treated groups when compared to the diabetic control group.

Effect of n-butanol fraction of *Alchornea cordifolia* on serum lipid profile in streptozotocin-induced diabetic Wistar rats:

Table 2 shows the mean total cholesterol, triglyceride, high density-lipoprotein and low density-lipoprotein values in all experimental and control groups respectively. There was a statistically significant (P<0.05) rise in serum total cholesterol, triglyceride, and low density-lipoprotein and significant reduction (P<0.05) in serum level of high density-lipoprotein in the diabetic control group when compared to normal control animals. *Alchornea cordifolia* administration significantly decreased (p<0.05) serum total cholesterol, triglyceride and low density-lipoprotein levels and increased serum high density-lipoprotein level in the streptozotocin-induced animals at all doses and this was comparable to the response seen with glibenclamide treatment at the doses tested.

**Table 1:** Effect of n-butanol fraction of *Alchornea cordifolia* on blood glucose level in streptozotocin-induced diabetic Wistar rats

<table>
<thead>
<tr>
<th>Treatment Given</th>
<th>Blood glucose levels (mg/dl)</th>
<th>Day 1</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td></td>
<td>91.4±3.07</td>
<td>88.6±3.61</td>
<td>90.0±4.11</td>
<td>87.8±3.85</td>
<td>88.6±2.03</td>
</tr>
<tr>
<td>Diabetic control</td>
<td></td>
<td>381.0±55.75</td>
<td>383.4±29.85</td>
<td>384.0±26.93</td>
<td>363.8±18.03</td>
<td>364.4±17.79</td>
</tr>
<tr>
<td>n-But 200 mg/kg b w</td>
<td></td>
<td>382.4±42.01</td>
<td>282.2±29.03</td>
<td>203.0±15.55</td>
<td>154.2±18.47</td>
<td>114.0±12.87</td>
</tr>
<tr>
<td>n-But 400 mg/kg b w</td>
<td></td>
<td>372.6±20.29</td>
<td>246.6±19.07</td>
<td>185.8±20.21</td>
<td>140.0±13.11</td>
<td>102.2±5.03</td>
</tr>
<tr>
<td>n-But 800 mg/kg b w</td>
<td></td>
<td>382.6±32.57</td>
<td>260.8±24.95</td>
<td>175.8±16.73</td>
<td>120.8±6.29</td>
<td>104.8±3.43</td>
</tr>
<tr>
<td>Glibenclamide 10mg/kg b w</td>
<td></td>
<td>358.6±13.85</td>
<td>187.0±19.57</td>
<td>129.0±3.87</td>
<td>103.0±1.95</td>
<td>95.8±1.75</td>
</tr>
</tbody>
</table>

*Values are presented as mean ± SEM*  
*Values are statistically significant compared to control group at *p* < 0.05 *b* < 0.01 and *c*<0.001 while ns =not significant*

Effect of n-butanol fraction of *Alchornea cordifolia* on serum liver enzymes in streptozotocin-induced diabetic Wistar rats:
Table 3 shows the mean aspartate aminotransaminase (AST), alanine transaminase (ALT) alkaline phosphatase (ALP), total and conjugated bilirubin values in all experimental and control groups respectively. There was a statistically significant (p<0.05) rise in the level of serum AST, ALT, ALP, total and direct conjugated bilirubin in the diabetic control group compared to the non diabetic control animals. However, the study revealed the plant extract at doses tested significantly decreased (p<0.05) serum level of AST, ALT and ALP when compared with diabetic control group.

| Table 2: Effect of n-butanol fraction of Alchornea cordifolia on serum lipid profile in streptozotocin-induced diabetic Wistar rats |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Treatment Given                | Serum cholesterol (mg/dl) | total Triglyceride (mg/dl) | Serum HDL (mg/dl) | Serum LDL (mg/dl) |
| Normal control                 | 79.29±1.65       | 89.00±8.18       | 45.48±482        | 16.01±3.58      |
| Diabetic control               | 164.63±6.60      | 206.23±6.02      | 15.39±2.90       | 107.98±7.10     |
| n-But200mg/kg b w              | 135.57±5.00      | 159.33±4.46      | 22.92±3.27       | 80.78±5.57      |
| n-But400mg/kg b w              | 108.64±7.10      | 117.00±6.40      | 29.79±4.58       | 55.44±6.47      |
| n-But800 mg/kg b w             | 91.73±9.36       | 107.99±7.08      | 34.61±3.67       | 35.56±7.34      |
| Glibenclamide 10 mg/kg b w     | 105.42±9.57      | 107.41±16.40     | 35.14±4.13       | 48.79±6.17      |

Values are presented as mean ± SEM
Values are statistically significant compared to control group at p < 0.05

| Table 3: Effect of n-butanol fraction of Alchornea cordifolia on serum liver enzymes in streptozotocin-induced diabetic Wistar rats |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|
| Treatment Given                | ALT (IU/L) | AST (IU/L) | ALP (IU/L) |
| Normal control                 | 40.40±2.24  | 145.92±2.40  | 37.37±2.74   |
| Diabetic control               | 99.00±3.44   | 220.60±10.04  | 96.23±4.26   |
| n-But200 mg/kg b w             | 64.66±5.58   | 166.10±6.02   | 56.73±6.05   |
| n-But400 mg/kg b w             | 55.68±6.58   | 143.41±3.78   | 31.97±3.33   |
| n-But800 mg/kg b w             | 44.50±2.18   | 143.50±11.56  | 58.16±3.68   |
| Glibenclamide 10 mg/kg b w     | 46.80±6.44   | 133.64±6.06   | 39.37±8.00   |

Values are presented as mean ± SEM
Values are statistically significant compared to control group at p < 0.05

Discussion:

Diabetes mellitus is a serious risk factor for the development of multiple organ damage as a result of multiple and complex mechanisms. The intention of this study was to evaluate the ability of Alchornea cordifolia to protect against changes that occur in the liver and lipid abnormalities which is one of the most common metabolic complications of diabetes mellitus which is found in about 40% of diabetics (Ravi et al., 2005; Eze et al., 2012). Administration of Alchornea cordifolia leaf extract to the diabetic animals significantly reduced the blood glucose level in the group treated with 200mg/kg b w with a greater decrease recorded with 400 and 800mg/kg b w. when compared to the diabetic control group. Preliminary phytochemical screening of n-butanol fraction of leaf extract of Alchornea cordifolia revealed the presence of flavonoids and saponins among other secondary metabolites. Flavonoids have been reported to exert its anti-diabetic activity by inhibiting sodium-dependent glucose transporter Isoform 2 (Glut 2), the intestinal transporters for glucose, therefore it is also likely that it might reduce blood glucose level by inhibiting the glucose absorption from the intestine with consequent reduction in the blood glucose concentration as was observed in this present work (Song et al., 2002). The levels of serum triglyceride and cholesterol are usually elevated in diabetic patients (Patel, 2008). The hyperlipidemia is mainly occurs as a result of insulin deficiency and thereby affecting metabolic processes like lipolysis and lipogenesis (Arner, 2005). The result of the present study showed increased serum level of cholesterol, triglyceride, low-density and depleted serum concentration of high-density lipoprotein in streptozotocin treated animals. This results support the findings of Mendez and Balderas (2001) who reported increased plasma cholesterol, triglycerides, LDL-C and decreased HDL-C in streptozotocin-induced hyperglycemia in rats. Alchornea cordifolia administration significantly decreased serum total cholesterol, triglyceride and low-density-lipoprotein levels and increased high density-lipoprotein in the STZ-induced animals at all doses and this was comparable to the response seen with glibenclamide treatment at the doses tested. However, this beneficial effect of the plant extract on the lipid profile may be secondary to glycemic control. Hence, the stabilization of serum triglyceride and cholesterol levels in rats by the plant extract may be attributed to glucose utilization and hence depressed mobilization of fat (Iweala and Oludare, 2011). AST and ALT are important and critical enzymes in the biological processes (Maiti et al., 2004). Measurements of serum ALT and AST are used in the evaluation of liver damage. Elevation of these enzyme activities is considered as
Evidence for hepatic damage (Schindhelm et al., 2006). Consequently, they are considered specific indicators for liver damage (El-demerdash et al., 2002). In our study, there was a significantly elevated serum ALT, AST, ALP and total and direct conjugated bilirubin activities of diabetic rats when compared with the normal control rats. The significantly decreased activities of these enzymes upon treatment with various doses of the plant extract showed the protective effect of the extract against liver damage and therefore can improve prognosis of diabetic patients.

Conclusion:

In conclusion, the present study demonstrated the antidiabetic actions of Alchornea cordifolia through its anti-hyperlipidemic and hepatoprotective effects, hence lend credence to the traditional use of this plant in the management of diabetes mellitus and its secondary complications.

Reference


