Insulinomimetic, Antihyperlipidemic and Antioxidative Properties of *Azadirachta indica*. Possible Mechanism of Action

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Authors’ contributions

This work was carried out in collaboration between all authors. Author ASA designed the study and wrote the protocol. Author TIA performed the statistical analysis. Authors ASA, TIA and KJP wrote the first draft of the manuscript. Authors ASA, TIA, KJP, AI and BBA managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Currently available synthetic drugs for management of diabetes mellitus have been reported to cause side effects, hence searches for safer, readily available and cheaper sources among medicinal plants. One hundred and thirty rats weighing average of 94g were used for the studies. In the first study, *Azadirachta indica* (AZI) was assessed for its hypolipidemic property, in the second AZI was assessed for its antihyperglycaemic potential while in the third study; it was assessed for its antidiabetic, antilipidemic and antioxidative properties. The potency of 2 different doses of AZI was compared with that of glibenclamide (a reference drug). AZI exhibited hypoglycemic, antihyperglycemic, antihyperlipidemic and antioxidative properties which were comparable to that of glibenclamide. Different doses of AZI reversed weight loss due to...
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streptozotocin-induced diabetes mellitus. AZI extract caused increased synthesis of liver glycogen when compared with diabetic rats. Conclusively, antidiabetic property of AZI may imitate blood glucose regulatory action of insulin.

Keywords: Diabetes; hypoglycaemia; hyperlipidemia; glibenclamide; streptozotocin; Azadirachta indica.

1. INTRODUCTION

Diabetes mellitus is one of the most metabolic disorders that has caused significant morbidity and mortality due to microvascular (retinopathy, neuropathy and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) [1]. Diabetes is associated with impaired glucose metabolism that leads to increase in free radical production and increase in triglyceride and lipoprotein levels [2]. Oxygen free radical can start lipid peroxidation which can result to stimulation of glycation of proteins, inactivation of antioxidants enzymes leading to complications of diabetes [3]. The disease is spreading fast around the world and according to World Health Organization, diabetic population is likely to increase to 300 million or more by year 2025 [4]. Because various synthetic drug there are presently being used in the management of diabetes have been reported to have adverse effects, studies have been intensified to search for more effective but safer therapies. In addition, It has been reported that the percentage of deaths attributable to high blood glucose or diabetes that occurs prior to age 70 is higher in low- and middle-income countries than in high-income countries [5], hence, a reason to search for alternative but cheaper and effective means of managing diabetes which may be affordable for people of these regions.

Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylurea, biguanides and glinides. Many of them have a number of serious adverse effects; therefore, the search for more effective and safer hypoglycemic agents is one of the important areas of investigation. Over the last few years, researchers have aimed at identifying and validating plants derived substances for the treatment of various diseases. Interestingly, it is estimated that more than 25% of modern medicines are directly or indirectly derived from plants. Not quite long ago, some medicinal plants have been reported to be useful in diabetes worldwide and have been used empirically in antidiabetic and antihyperlipidemic remedies. Antihyperglycemic activity of the plants is mainly due to their ability to restore the function of pancreatic tissue by causing an increase in insulin output or inhibit the intestinal absorption of glucose or to the facilitation of metabolites in insulin-dependent process.

**Azadirachta indica** is a fast growing, evergreen tree found commonly in India, Africa and America. The importance of the Neem tree has been recognized by the US National Academy of Sciences, which published a report in 1992 entitled ‘Neem – a tree for solving global problems’. The advancement of Neem research has earlier been documented [6]. Neem has found to contain a vast array of biologically active compounds, which are chemically diverse and have got an enormous therapeutic potential. German researchers have proven Neem extracts prevent tooth decay and periodontal disease [7,8] leading to good oral health [9]. Neem leaf extract has a antimicrobial effect on *Enterococcus faecalis* and *Candida albicans*. Therefore, it can be a potential endodontic irritant [10]. It has been reported to have antifertility [11], antibacterial [12], and antimalarial [13] properties. The present study assessed possible antidiabetic properties of *A. indica*.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Albino rats with an average weight of 194 g were purchased from commercial breeder in Ilorin, Kwara State. They were kept in a well-ventilated cage in the animal house of the\ Department of Anatomy, Ladoke Akintola University of Technology, Nigeria. The animals have unrestricted access to clean water and were fed commercial normal feed. They were separated into groups according to the experimental procedures, each group consisting of ten rats.

2.1.1 Ethical consideration

All animal procedures were in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals [14] as well as Ethical Guideline for The Use of Laboratory Animals in LAUTECH, Nigeria.
2.2 Plant Materials and Extract Preparation

Leaves of *Azadirachta Indica* were obtained in Ogbomoso, Nigeria and was identified by Dr. Ogunkunle of the Botany Unit, Department of Pure and Applied Biology and were confirmed with a plant name index. The leaves were thoroughly washed with water. The leaves were dried in room temperature and grinded into a powdery form using electric grinding machine. The powder sample of *A. Indica* (650 g) was soaked in ethanol (98%) for 3 days at room temperature. Thereafter, the soaked powder was further macerated. The combined ethanolic extract was filtered and the solvent was fully evaporated under reduced pressure using soxhlet extractor apparatus.

2.3 Hypoglycemic Effect of Ethanol Extract of *Azadirachta indica* (AZI) in Normal Rats

Forty rats were made to fast overnight and were divided into four groups of ten animals each. The first group served as a control group and they received 4 ml/kg body weight of distilled water. Rats in groups II and III received 100 and 200 mg/kg of AZI respectively. Rats in group IV received 600 µg/kg of Glibenclamide (GLB) (reference standard drug) [15,16]. The baseline fasting blood glucose was determined before oral administration of respective treatment.

2.4 Hypoglycemic Effect of AZI in Glucose-loaded Normal Rats

Forty rats were made to fast overnight and were divided into four groups of ten animals each. The first group served as a control group and they received 4 ml/kg body weight of distilled water. Rats in groups II and III received 100 and 200 mg/kg of AZI respectively. Rats in group IV received 600 µg/kg of Glibenclamide (GLB) (reference standard drug). The treatment was administered orally, 30 min before the glucose load (2 g/kg) [17]. Blood samples were taken before and 30, 60 and 120 min after glucose intake and analyzed for glucose level [18].

2.5 Assessment of antidiabetic Property of AZI

2.5.1 Induction of type I diabetes

Fifty rats were used for this study. They were divided into five groups of ten rats each. A single dose of streptozotocin (40 mg/kg, ip) was administered to Groups II, III IV and V rats. While normal control rats (group I) were injected with saline alone. Streptozotocin was dissolved in freshly prepared 0.1M citrate buffer (pH 4.5) (Animal model of diabetes). Streptozotocin-injected animals were given 5% glucose for 24 h to prevent initial streptozotocin induced hypoglycemic mortality [19].

2.5.2 Experimental protocol for assessment of antidiabetic, antihyperlipidemic and antioxidative property of AZI

The animals were randomly divided into five groups of eight rats each. Groups I and II served as normal control and diabetic control treated with 4 ml/kg of distilled water, respectively. Groups III and IV diabetic rats were administered 100 and 200 mg/kg of AZI respectively. Rats in group V received 600 µg/kg of Glibenclamide (GLB) (reference standard drug). The daily oral treatment was administered in between 08.00 to 09.00 h for 15 days.

2.5.3 Preparation of glibenclamide suspension

Suspensions of glibenclamide as standard drug were prepared using 0.3% w/v sodium carboxymethylcellulose in distilled water.

2.6 Analysis of Biochemical Parameters

2.6.1 Determination of fasting blood glucose concentration

To determine this, the animals were made to fast overnight. In the morning before they were given access to meal, the tip of the tail of each of them was cut with dissecting scissors and blood was allowed to drop on the glucometer strip that was already loaded into the glucometer. The value of Fasting Blood Glucose was displayed on the screen of the glucometer.

2.6.2 Determination of malondialdehyde (MDA) in liver tissue

Lipid peroxidation was assessed by measuring the formation of thiobarbituric acid reactive substances (TBARS) using the procedure of [20]. The assay was based on the reaction of chromogenic reagent (2 – TBA) with MDA (end product of lipid peroxidation) under acidic condition to yield a stable pink chromophore with maximum absorbance at 532 nm. Briefly, to 0.3 ml of sample, 1.2 ml of 5% TCA was added and
the mixture was centrifuged at 4000 rpm for 15 minutes. The supernatant was decanted. To 1 ml of supernatant, 2 ml of TBA solution was added and this was boiled for 10 minutes and cool at room temperature. Absorbance was read at 532 nm.

2.6.3 Determination of lipids and lipoprotein

Total cholesterol was determined using enzymatic method described by [21]. Triglyceride was determined using enzymatic method described by [22]. The precipitation method by [23] and total cholesterol determination method by [21] were used to determine HDL cholesterol.

2.6.4 Assessment of lipid peroxidation

Lipid-peroxidation was assessed by measuring the thiobarbituric acid reactive (TBAR) products using the procedure of [24] and expressed as nanomole/ milliliter serum.

2.6.5 Determination of superoxide dismutase (SOD) activity

The level of superoxide dismutase (SOD) activity was determined by the method of [25].

2.6.6 Determination of catalase activity

Catalase activity was determined according to the method of [26].

3. RESULTS

Streptozotocin-induced diabetes caused significant (P<0.05) reduction in body weight of rats, however, there was increased body weight in diabetic rats treated with AZI in a dose-dependent manner (Table 1). GLB and both dosages of Azadirachta indica caused comparable significant (P<0.05) increase in body weight when compared with diabetic rats while the diabetic group exhibited significant (P<0.05) reduction in body weight at 7, 10 and 15 days. In the same vein, treatment with AZI exhibited hypoglycaemic property by reversing high serum glucose concentration due to ingestion of 2 g/kg load of glucose in a dose-dependent fashion (Table 3). In this same study, we used the estimation of total cholesterol, triglyceride, LDL-cholesterol and HDL-cholesterol to evaluate the antihyperlipidemic potential of Azadirachta indica’s phenolic extract in both normal and diabetic control groups and the results were presented as Tables 4a and 4b. Administration of AZI extract, at both dosages significantly (P<0.05) reduced total cholesterol, triglyceride and LDL-C concentrations when compared with corresponding values in the untreated diabetic groups after 21 days. These reductions are comparable with that observed in diabetic rats treated with GLB. In the same vein, AZI caused elevated serum concentration of HDL-C. At 7, 10 and 15 days. Streptozotocin-induced diabetes induced oxidative stress as indicated by elevated concentration of marker of lipid peroxidation (MDA) as well as reduction in activities of enzymic markers (Catalase and Superoxide dismutase. AZI extract exhibited antioxidative property by inducing enhanced activities of catalase and superoxide dismutase as well as reducing lipid peroxidation as indicated by reduced MDA concentration (Table 5).

Administration of AZI (100 and 200 mg/kg) and glibenclamide caused observable reduction in blood glucose on successive days of the experiment as compared to their basal values. The reductions were significant (P<0.05) on days 7, 10 and 15.

Table 1. Effects of Azadirachta indica on body weight of rats induced with diabetes

| Treatment | Normal group | Diabetic group | AZI (100 mg/kg) | AZI (200 mg/kg) | GLB |
|-----------|--------------|----------------|----------------|----------------|-----|
| Basal value | 195.40±4.0 | 193.35±3.6 | 196.23±3.3 | 195.05±4.3 | 190.22±5.1 |
| After treatment | | | | | |
| 1 | 197.25±1.2 | 188.38±3.3 | 197.35±3.5 | 197.12±2.4 | 192.08±1.8 |
| 4 | 198.75±1.0 | 184.21±3.1 | 199.76±2.3 | 199.53±2.2 | 194.88±2.3 |
| 7 | 203.12±0.9 | 181.23±2.7 | 204.41±4.2 | 203.36±2.0 | 198.35±1.7 |
| 10 | 206.52±1.1 | 175.27±2.5 | 208.23±3.3 | 207.51±4.3 | 203.06±4.1 |
| 15 | 209.16±2.0 | 166.16±1.9 | 213.17±3.3 | 210.01±2.8 | 207.17±3.9 |

Body weight was expressed is gramme and each value is presented as mean ±SD. ps0.05 is regarded as level of significance. aStatistically different from diabetic rats; bStatistically different from non-diabetic rat
Glucose loaded rats showed significant elevation (P<0.05) in fasting blood glucose on successive minutes (30, 60 and 120) of the experiment as compared to their basal values. Administration of AZI (100 and 200 mg/kg) and glibenclamide caused significant reduction (P<0.05) in blood glucose on successive minutes of the experiment as compared to values at 30 minutes.

### 3.1 Effect of AZI on Serum Lipid Profile of Diabetic Rats

The levels of cholesterol, triglycerides and LDL-C were significantly (p<0.05) increased in streptozotocin-induced diabetes while HDL levels decreased significantly (p<0.05), when compared with normal control (Tables 4a and b). Treatment with AZI and standard drug (glibenclamide) significantly (p<0.05) lower the blood levels of cholesterol, triglycerides and LDL-C but caused significant (p<0.05) increase in HDL-C levels, when compared with diabetic control groups.

#### 3.1.1 Effect of AZI on antioxidant activity in diabetic rats

STZ-induced diabetes caused lipid peroxidative damage with reduced activities of catalase and superoxide dismutase. However, both the AZI and reference drug significantly (p<0.05) reduced altered lipid peroxidative damage and improved activities of the antioxidative enzyme. The lowest activities of CAT and SOD was seen in the diabetic rats (Table 5). 200 mg/kg phenolic extract of *Azadirachta indica* and GLB showed significant reduction of malondialdehyde concentration when compared with non-diabetic and diabetic control groups while 100 mg/kg dosage only showed a significant (P<0.05) reduction when compared with the diabetic control group. Catalase concentration for both dosages and GLB were significantly (P<0.05) increased when compared with diabetic and non-diabetic groups while 100 mg/kg and 200 mg/kg significantly (P<0.05) increased superoxide dismutase concentration when compared with non-diabetic groups.

### Table 2. Hypoglycemic effect of AZI in normal rats

| Treatment | Normal | AZI (100mg/kg) | AZI (200mg/kg) | GLB |
|-----------|--------|----------------|----------------|-----|
| Basal value | 79.12±0.3 | 76.09±1.2 | 79.35±1.0 | 77.17±0.2 |
| After treatment | | | | |
| 1 | 78.41±0.5 | 73.93±0.9 | 71.42±1.2 | 72.51±0.5 |
| 4 | 76.25±1.2 | 72.33±0.3 | 65.18±0.8 | 65.31±1.2 |
| 7 | 77.45±0.4 | 68.16±1.5b | 60.25±0.6b | 56.09±0.9b |
| 10 | 78.22±0.2 | 62.36±0.7b | 53.16±1.0b | 47.15±1.4b |
| 15 | 78.91±1.7 | 55.21±1.2b | 45.48±0.7b | 37.06±0.5b |

Blood glucose was expressed in mg/dl and each value is presented as mean ±SD. p ≤ 0.05 is regarded as level of significance. bStatistically different from non-diabetic rats.

### Table 3. Hypoglycemic effect of AZI after glucose load

| Treatment | Normal control | AZI (100mg/kg) | AZI (mg/kg) | GLB |
|-----------|----------------|----------------|-------------|-----|
| Basal value | 79.34±1.1 | 80.25±0.7 | 78.91±1.3 | 79.54±0.9 |
| After glucose loading | | | | |
| 30 mins | 131.3±1.1a | 132.2±0.9a | 130.7±1.2a | 133.24±1.3a |
| 60 mins | 122.1±0.9a | 120.4±1.6a | 111.3±0.8a | 106.17±0.7a |
| 120 mins | 110.2±1.3a | 109.1±0.7a | 101.4±0.6a | 93.51±0.9a |

Blood glucose was expressed in mg/dl and each value is presented as mean ±SD. p ≤ 0.05 is regarded as level of significance. aSignificantly different from their basal values.

### Table 4a. Antihyperlipidemic effects of AZI in diabetic rats

| Total cholesterol | Triglyceride |
|-------------------|--------------|
| Normal control | 83.44±0.95 | 81.17±0.45 |
| Diabetic control | 85.39±2.10 | 103.15±1.51c |
| AZI (100 mg/kg) | 91.23±0.45 | 83.16±0.25a |
| AZI (200 mg/kg) | 88.40±1.05 | 85.00±6.16a |
| GLB | 84.05±0.25 | 72.00±2.91ac |

Total cholesterol and triglyceride were expressed in mg/dl and each value is presented as mean±SD. acSignificantly different from day 0 value. aSignificantly different from diabetic control
Table 4b. Antihyperlipidemic effects of AZI in diabetic rats

|          | HDL-C |           | LDL-C |           |
|----------|-------|-----------|-------|-----------|
|          | Day 0 | Day 21    | Day 0 | Day 21    |
| Normal control | 19.00±1.20 | 18.95±0.79 | 51.98±2.10 | 48.57±1.70 |
| Diabetic control | 18.45±0.79 | 7.90±0.90 | 53.49±1.75 | 57.01±4.12 |
| AZI (100 mg/kg) | 18.85±1.22 | 24.85±1.35 | 60.35±2.05 | 49.01±3.15 |
| AZI (200 mg/kg) | 17.36±0.91 | 19.00±1.04 | 52.94±1.80 | 40.10±2.90 |
| GLB | 18.29±1.45 | 24.30±0.95 | 49.70±3.15 | 29.78±2.10 |

HDL-C and LDL-C were expressed in mg/dl and each value is presented as mean± SD

Significantly different from day 0 value, *Significantly different from diabetic control

Table 5. Antioxidative effects of AZI in diabetic rats

|          | Lipid peroxidation (nmol/ml serum) | Catalase (iu.mg/protein) | Sod unit (iu.mg/protein) |
|----------|-----------------------------------|--------------------------|--------------------------|
| Normal control | 0.57±0.03a | 140.20±4.2a | 0.24±0.01a |
| Diabetic control | 6.35±1.4 | 15.35±1.2 | 0.05±0.03 |
| AZI (100 mg/kg) | 0.58±0.01a | 115.35±1.2 | 0.30±0.01a |
| AZI (200 mg/kg) | 1.57±0.37ac | 101.25±16.1 | 0.17±0.02ae |
| GLB | 0.77±0.02ac | 118.41±3.4 | 0.21±0.06d |

Each value is presented as mean± SD. *Significantly different from diabetic control

Significantly different from non-diabetic rats

3.1.2 Effect of AZI on blood glucose level in diabetic rats

Diabetes induction using STZ showed significant hyperglycaemia in diabetic groups. Oral administration of the AZI (100 and 200 mg/kg) and the standard drug (glibenclamide) for 21 days significantly (p<0.05) lowered blood glucose levels (Table 6). The effect of AZI on blood glucose was dose-dependent.

4. DISCUSSION

While the inability of β-cells of the islet of langerhans of the pancreas to secrete enough insulin for the homeostatic control of blood glucose level has been firmly established as the sole brain behind the menace of type-1 diabetes, this same reason has been known to contribute immensely to the onset and progression of its type-2 counterpart. It is well known that folk medicine presents innumerable measures for the management of diabetes and its downstream complications; however, the key mechanisms surrounding their antidiabetic strength are not well understood [27]. One of the mechanisms exploited by these bioactive compounds is their ability to regulate glucose metabolism and transport as well as to induce insulin release from β-cells in the islet of langerhans of the pancreas [28,29,30]. In this study, we investigated the hypoglycemic effect of ethanolic extract of *Azadirachta indica* in both normal and streptozotocin-induced diabetic rats.

Table 6. Antidiabetic effects of AZI in diabetic rats

|          | Pre-STZ | Day 0 | Day 7 | Day 14 | Day 21 |
|----------|---------|-------|-------|--------|--------|
| Normal control | 92.34±2.60 | 96.12±3.24 | 101.12±5.34 | 108.33±8.54 | 114.43±7.45 |
| Diabetic control | 86.22±5.31 | 312.47±82.63 | 344.65±87.43 | 370.41±120.43 | 394.13±101.28 |
| AZI (100 mg/kg) | 82.62±3.61 | 239.44±41.25 | 255.23±21.54 | 281.74±52.22 | 335.67±24.15 |
| AZI (200 mg/kg) | 90.56±12.31 | 360.43±65.22 | 271.31±26.11 | 243.67±31.56 | 160.25±12.04 |
| GLB | 87.57±14.21 | 325.42±54.44 | 276.36±28.74 | 154.21±13.54 | 134.78±21.66 |

Blood glucose was expressed in mg/dl and each value is presented as mean ±SD. p≤0.05 is regarded as level of significance. *Significantly different from diabetic control **Significantly different from non-diabetic rats.
The liver associated with decreased high-density lipoprotein cholesterol (HDL-C) in circulation [34,35,36]. Overproduction of triglyceride-rich lipoproteins in (hypercholesterolemia) and HDL-C, LDL-C, atherogenic free fatty acids, triglycerides, and lipoprotein lipase activities have been explained to be one of the reasons behind the prevalence of Insulin Dependent Diabetes Mellitus (IDDM) [37]. In the present study, STZ induced diabetic rats exhibited high concentrations of total cholesterol, triglyceride, LDL-cholesterol and low concentration of HDL-cholesterol. This is in agreement with previous studies where heterogeneous hyperlipidemia was reported [38,39]. Excessive serum levels of cholesterol and triglyceride have been reported to be involved in the induction of coronary heart disease and atherosclerosis, which are the secondary complications occurring in the diabetes [40]. However, administration of different doses of A. indica reduced the levels of TC, TG, and LDL-C to near normal comparable to what was observed in the non-diabetic rats. Similar results were observed in diabetic rats treated with GLB. The reason for this ameliorative effects could be that AZI improved insulin synthesis which in turn may inhibit hormone sensitive lipase and increase the utilization of glucose and decrease the mobilization of free fatty acids from the fat depositions [37]. Hypertriglyceridemia in diabetes occurs, in part, because insulin action regulates lipid flux. Insulin promotes the activity of the enzyme lipoprotein lipase, which mediates free fatty acid uptake into adipose tissue (storage) and also suppresses the activity of the enzyme hormone-sensitive lipase, resulting in decreased release of free fatty acids into the circulation [38]. The HDL is involved in the transport of cholesterol from peripheral tissues to the liver, thereby acting as a protective factor. In this study, level of HDL-C was decreased in STZ-induced diabetic rats. However, AZI at the different dosages was able to cause increase in the serum concentration of HDL-C in the diabetic rats. The implication of this is that AZI may enhance reverse cholesterol transport i.e. cholesterol transport from the peripheral tissues to the liver, hence preventing excessive blood cholesterol level.

Oral glucose tolerance test is performed to assess ability of body system to cope with increased blood glucose by regulating blood glucose after ingestion of high carbohydrate meal and this exposes altered carbohydrate metabolism during post glucose administration. The ability of ethanolic extract of AZI to lower the blood glucose level in oral glucose tolerance test suggest that ethanolic extract of AZI enhances better glucose utilization capacity [32]. The glucose lowering effects could possibly be that ethanolic extract of AZI induced increased insulin synthesis or improved glucose transportation and consumption by the body [33]. Although, the glucose lowering effect of 100 mg/kg of AZI was similar to the non-treated rats, however, that of the 200 mg/kg was higher. This suggests that ethanolic extract of AZI may have better blood glucose regulating ability at higher doses.

Hyperlipidemia comprises of heterogeneous group of disorders which are characterized by high levels in one or more lipids and/or lipoproteins. These lipids and lipoproteins include atherogenic free fatty acids, triglycerides, LDL-Cholesterol (hypercholesterolemia) and HDL-Cholesterol (HDL-Cholesterol) in circulation [34,35,36]. Overproduction of triglyceride-rich lipoproteins in the liver associated with decreased high-density lipoprotein cholesterol levels have been seen in patients with non Insulin Dependent Diabetes Mellitus (NIDDM) while increased adipose tissue and lipoprotein lipase activities have been explained to be one of the reasons behind the prevalence of Insulin Dependent Diabetes Mellitus (IDDM) [37]. In the present study, STZ induced diabetic rats exhibited high concentrations of total cholesterol, triglyceride, LDL-cholesterol and low concentration of HDL-cholesterol. This is in agreement with previous studies where heterogeneous hyperlipidemia was reported [38,39]. Excessive serum levels of cholesterol and triglyceride have been reported to be involved in the induction of coronary heart disease and atherosclerosis, which are the secondary complications occurring in the diabetes [40]. However, administration of different doses of A. indica reduced the levels of TC, TG, and LDL-C to near normal comparable to what was observed in the non-diabetic rats. Similar results were observed in diabetic rats treated with GLB. The reason for this ameliorative effects could be that AZI improved insulin synthesis which in turn may inhibit hormone sensitive lipase and increase the utilization of glucose and decrease the mobilization of free fatty acids from the fat depositions [37]. Hypertriglyceridemia in diabetes occurs, in part, because insulin action regulates lipid flux. Insulin promotes the activity of the enzyme lipoprotein lipase, which mediates free fatty acid uptake into adipose tissue (storage) and also suppresses the activity of the enzyme hormone-sensitive lipase, resulting in decreased release of free fatty acids into the circulation [38]. The HDL is involved in the transport of cholesterol from peripheral tissues to the liver, thereby acting as a protective factor. In this study, level of HDL-C was decreased in STZ-induced diabetic rats. However, AZI at the different dosages was able to cause increase in the serum concentration of HDL-C in the diabetic rats. The implication of this is that AZI may enhance reverse cholesterol transport i.e. cholesterol transport from the peripheral tissues to the liver, hence preventing excessive blood cholesterol level.

In Streptozotocin (STZ) induced diabetes group rats, there is a loss in body weight probably due to muscle destruction or degradation of structural proteins [31]. Different doses of ethanolic extract of AZI and glibenclamide significantly improve the body weight when compared to the diabetic rats. This may be an indication that both ethanolic extract of AZI and glibenclamide possess protective property in controlling muscle wasting (reversal of gluconeogenesis). It is important to note that the effect is dose dependent.

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Hyperlipidemia comprises of heterogeneous group of disorders which are characterized by high levels in one or more lipids and/or lipoproteins. These lipids and lipoproteins include atherogenic free fatty acids, triglycerides, LDL-Cholesterol (hypercholesterolemia) and HDL-Cholesterol (HDL-Cholesterol) in circulation [34,35,36]. Overproduction of triglyceride-rich lipoproteins in the liver associated with decreased high-density lipoprotein cholesterol levels have been seen in patients with non Insulin Dependent Diabetes Mellitus (NIDDM) while increased adipose tissue and lipoprotein lipase activities have been explained to be one of the reasons behind the prevalence of Insulin Dependent Diabetes Mellitus (IDDM) [37]. In the present study, STZ induced diabetic rats exhibited high concentrations of total cholesterol, triglyceride, LDL-cholesterol and low concentration of HDL-cholesterol. This is in agreement with previous studies where heterogeneous hyperlipidemia was reported [38,39]. Excessive serum levels of cholesterol and triglyceride have been reported to be involved in the induction of coronary heart disease and atherosclerosis, which are the secondary complications occurring in the diabetes [40]. However, administration of different doses of A. indica reduced the levels of TC, TG, and LDL-C to near normal comparable to what was observed in the non-diabetic rats. Similar results were observed in diabetic rats treated with GLB. The reason for this ameliorative effects could be that AZI improved insulin synthesis which in turn may inhibit hormone sensitive lipase and increase the utilization of glucose and decrease the mobilization of free fatty acids from the fat depositions [37]. Hypertriglyceridemia in diabetes occurs, in part, because insulin action regulates lipid flux. Insulin promotes the activity of the enzyme lipoprotein lipase, which mediates free fatty acid uptake into adipose tissue (storage) and also suppresses the activity of the enzyme hormone-sensitive lipase, resulting in decreased release of free fatty acids into the circulation [38]. The HDL is involved in the transport of cholesterol from peripheral tissues to the liver, thereby acting as a protective factor. In this study, level of HDL-C was decreased in STZ-induced diabetic rats. However, AZI at the different dosages was able to cause increase in the serum concentration of HDL-C in the diabetic rats. The implication of this is that AZI may enhance reverse cholesterol transport i.e. cholesterol transport from the peripheral tissues to the liver, hence preventing excessive blood cholesterol level.

So far, it has become increasingly glare that oxidative stress is a key factor in the pathogenesis of diabetes mellitus [39]. Although, the animals and human studies already suggested that several factors might dictate the pathophysiology of diabetes mellitus, the role of oxidative stress through the uprising of reactive oxygen species (ROS) cannot be undermined.
The antioxidant enzyme system prevents/ reduces ROS production and enzymes involve include superoxide dismutase (SOD), catalase (CAT) and glutathione Peroxidase (GPX). Oxidative stress had also been reported to increase the concentration of 2-thiobarbituric acid reactive substances (TBARS) in which MDA is one of them [41]. In this study, streptozotocin-induced diabetes caused increased lipid peroxidation. It has been reported that increased glucose level induces overproduction of oxygen free radicals and consequently increases the protein and lipid oxidation [42]. Therefore, in this study, in order to verify and establish the antioxidative potential of Azadirachta indica, we focused on some critical biomarkers of oxidative stress which include malondialdehyde (MDA), superoxide dismutase and catalase. STZ-induced diabetic rats exhibited reduced serum superoxide dismutase and catalase. The reason for this may be that excessive generation of reactive oxygen species caused the enzymes to be excessively utilized thereby overwhelming the ability of the system to combat oxidation. However, administration of AZI at different doses caused increased levels of the two enzymes. Diabetic rats also showed increased level of malondialdehyde, a marker of fatty chain peroxidation. Treatment with different doses of AZI decreased the concentration of MDA. This may indicate that the antioxidative property of AZI may involve inhibition of lipid peroxidation and increase in antioxidant enzymes. Furthermore, many plants have been reported to contain phenolics and flavonoids and these bioactive components are known for their potent antioxidative properties. Phenolic compounds are considered strong antioxidants capable of chelating transition metals ions, preventing free radical chain reactions and oxidative damage [43]. Oxidative stress has been associated with the progression of several chronic diseases in humans. Scientific evidence from the literature has shown that elevated levels of reactive oxygen species (ROS) may induce oxidation of proteins, lipids and DNA [44], leading to the alteration of their normal functions. However, studies have shown that long term consumption of diets rich in plant polyphenols has been associated with reduced risk factor of cancer, cardiovascular diseases, diabetes and neurological disorders [45,46,47].

Streptozotocin-induced diabetes mellitus caused reduced concentration of hepatic glycogen. The implication may be that diabetes mellitus stimulates glycogenolysis leading to reduced glycogen concentration. However, the different doses of AZI caused increased concentration of hepatic glycogen. The levels of hepatic glycogen in AZI-treated rats are comparable to that seen in non-diabetic rats as well as in GLB treated rats. This may be suggestive that AZI stimulated both insulinotropic effect and glycogen synthase activity in the treated group. Although several studies have shown antidiabetic, antihyperlipidemic and antioxidant properties of Azadirachta indica, however, none of them have shown possible mechanism of how the excessive blood glucose i.e. hyperglucaemia might have been reduced. However, the highlight of this study is that the mechanism by which Azadirachta indica exhibited hypoglycaemic and antihyperglycaemic properties was is shown to be induction glycogenesis thereby supporting anabolism.

Therefore, the insulinomimetic, antihyperlipidemic and antioxidant potential of Azadirachta indica through its ability to reduce blood glucose level, regulate lipid metabolism, reduce malondialdehyde production and increase in vivo antioxidant status of diabetic rats could present a reasonable platform its antidiabetic relevance and its suggestion as a strong nutraceutical for the management/treatment of diabetes mellitus.

5. CONCLUSION

This study further affirms the antidiabetic property of Azadirachta indica. It also suggests the nutraceutical relevance of the medicinal plant and its insulin mimicking property.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All animal procedures were in strict accordance with the NIH Guide for the Care and Use of Laboratory Animals (13) as well as Ethical Guideline for The Use of Laboratory Animals in LAUTECH, Nigeria.

COMPETING INTERESTS

There is no conflict of interest as the study was not sponsored by any organization. All the authors agreed to the contents of the manuscript before it was sent out for publication.
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