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Phytochemistry and antidiabetogenic property of aqueous extract of *Azadirachta indica* leaf in streptozotocin-induced diabetic rats

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ABSTRACT

Aim: This study was carried out to determine the phytochemicals and the antidiabetogenic property of *Azadirachta indica* leaf in streptozotocin-induced diabetic male rats. **Methodology:** The quantitative phytochemical analysis and the glucose levels were checked using standard methods. Different group of rats were pre-treated for seven, fourteen, twenty-one and twenty-eight days before the induction of diabetes to know the extent of protection the extract will have on the rats by preventing the onset of diabetes. At the end of each treatment period diabetes was induced intraperitoneally using 50mg/kg bodyweight of Streptozotocin. **Results:** The percentage composition of the phytochemicals in the leaves of *Azadirachta indica* was as follows: Alkaloids (11.36%), cardiac glycosides (6.19%), flavonoid (5.34%), heamaglutinin (6.331%), oxalate (0.0204%), phenol (4.312%), phytate (4.600%), saponin (12.55%), steroid (0.2566) and tannin (6.17%). The result obtained revealed that there was a better protection for the group of rats pre-treated for a period of three weeks and four weeks. The percentage decrease in weight after the induction of diabetes for the groups pre-treated for a period of twenty-one days at varying doses of 100, 200 and 400 mg/kg bodyweight was 6.67%, 6.05% and 4.69% respectively which was significantly (p<0.05) different from the normal untreated group (9.90%). The percentage decrease in weight after the induction of diabetes for the groups pre-treated for a period of twenty-one days at varying doses of 100, 200 and 400 mg/kg bw is 7.04%, 5.97% and 2.50% respectively which was significantly (p<0.05) different from the normal untreated group (10.8%). The percentage increase in the fasting blood glucose level for the groups pre-treated for a period of twenty-one days at varying doses of 100, 200 and 400 mg/kg bw was 65.4%, 76.3% and 72.4% respectively which is significantly (p<0.05) different from that of the normal untreated group (82.7%) and the group pre-treated with metformin at a dose of 100 mg/kg bw (84.5%). The percentage increase in the fasting blood glucose level for the groups pre-treated for a period of twenty-eight days at varying doses of 100, 200 and 400 mg/kg bw was 73.5%, 68.5% and 64.4% respectively which was significantly (p<0.05) different from that of the normal untreated group (83.3%) and the group pre-treated with metformin at a dose of 100 mg/kg bw (85.5%). The longer the period of pre-treatment, the better the reduction in the blood glucose levels recorded after the induction of diabetes. **Conclusion:** These results suggest that the aqueous extract of *A. indica* leaf has antidiabetogenic effect and could be used in the prevention as well as delaying the onset of diabetes mellitus.

Keywords: Phytochemistry, Antidiabetogenic, *Azadirachta indica*, Streptozotocin, Diabetic rats.

1. INTRODUCTION

Diabetes mellitus (DM) is a rapid metabolic disorder which can result from a defect in insulin secretion, insulin action, or both. Insulin deficiency in turn leads to chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism [1]. It is one of the chronic non-communicable diseases which have emerged as a leading global health challenge. It is a risk factor for vascular brain diseases, renal failure, blindness and diabetic wound [2]. During the progression of the disease the body cannot breakdown glucose properly as a result of insufficient insulin or when the insulin produced is not effectively used by the body leading to high blood glucose level. The two main type of diabetes are type 1 diabetes and type 2 diabetes. Type 1 also known as insulin-dependent diabetes is characterized by insufficiency of the production of insulin as a result of damage of the beta cell of the pancreas [3]. Type 1 diabetic patients are likely to develop ketoacidosis. Type 2 diabetes known as non-insulin dependent diabetes is characterized by inefficient use of the insulin produced by the beta cells of the pancreas. 90% cases of diabetes is type 2 diabetes [4].

In 2017, it was estimated that 425 million people between the ages of 20–79 years suffered from DM. This figure is expected to rise to 629 million by 2045 [5]. In the year 2019 international diabetes federation

reported that about 463 million individuals are affected with diabetes worldwide and the number was expected to increase by the year 2030 to 578 million and by the year 2045 it was estimated to increase to 700 million [6]. In Africa, 19 million adults (20-79) are living with diabetes and it is estimated to increase to 47million by 2045, 45million adults (20-76) are at a high risk of developing type 2 diabetes because of impaired Glucose Tolerance and it is estimated to reach 110 million by the year 2045, 60% of adult living with diabetes are undiagnosed, and about 9.5 billion dollars has been spent on healthcare for people with diabetes [6]. Base on a random-effects metal-analysis carried out from the year 1990 to 2017, an increasing trend of about 440% in type 2 diabetes mellitus cases was observed in Nigeria in the year 2015 over the value gotten in 1990 which was 2.2% [7]. In Nigeria, the estimated values of prevalence of diabetes in the six geopolitical zone were 3.0%, 5.9%, 3.8%, 5.5%, 4.6%, and 9.8% in the north-west, north east, north central, south west, south east, and south respectively [8].

According to International Diabetes Federation, about 2 million cases of diabetes have not been diagnosed in Nigeria leading to over 40 thousand deaths as a result of the disease and its complications [9]. Nigerians have been known to use various parts of herbs to treat diseases [10]. These plants hold active compounds that are medicinal which are ingredient in drugs [11]. *Azadirachta indica* (neem) is a tropical tree belonging to meliaceae family, native to India [12]. *Azadirachta indica* is an important plant. The effect of the aqueous extract of *A. indica* leaf on some essential biochemical parameters has earlier been reported [13]. It is one of the most effective blood-purifiers, detoxifiers and immune system boosters. So many diseases have shown to respond to its therapeutic activity. *A. indica* leaf can be taken as tea. The oil can be applied externally or a few drops can be put in an empty capsule and taken internally. It enhances soil fertility [14]. About 72 countries grows *A. indica*trees, Africa included.

A. indica contains active compounds that are therapeutic. The main chemical compounds are terpene, lavonoids, carotenoid, nimbin, melicain (the bitter taste) gedunin, salanin, valassin [15]. The limnoids compound in *A. indica*possess insecticidal properties [16]. In Thailand the leave extract is used as an antioxidant for food and industries (pharmaceutical industry) [17, 18]. It is used in India to cure skin disease such as ringworm, scabies [19]. The mixture of neem and turmeric paste was applied on 184 individuals with scabies, 97% of the individuals got cured within 3 to 15 day of administration with no side effect [20]. The leaf extracts in diabetic induced rat improves the size, shape and number of beta cells thereby regenerating the beta cell [21]. The antidiabetogenic property of the leaves of *A. indica* in diabetic rats for a period of two weeks has earlier been studied. The result of the previous studies revealed that the longer the period of pre-treatment, the better the antidiabetogenic property [22]. The present study was carried out to determine the quantitative phytochemicals and antidiabetogenic property of aqueous extract of *A. indica* leaf in streptozotocin-induced diabetic rats at different periods (seven, fourteen, twenty-one and twenty-eight days) of pre-treatment.

2. MATERIALS AND METHODS

2.1 Sample Collection and Identification

The leaves of *A. indica* were collected at Nnamdi Azikiwe University Awka, Anambra State, Nigeria. The sample was identified and authenticated by a taxonomist in the Department of Botany, Nnamdi Azikiwe University, Awka. The voucher number as deposited in the herbarium of the Department of Botany, Nnamdi Azikiwe University, Awka, Nigeria is NAU/H/14.

2.3 Preparation of the Aqueous Extracts of Neem Leaf

The leaves were hand-picked, thoroughly washed and air dried at room temperature. The dried leaves were pulverized into powder using Corona manual grinding machine. One kilogramme of the pulverized leaves powder of *A. indica* was soaked in 3 litres of distilled water for 24 hrs for complete extraction. The aqueous extraction was sieved using muslin cloth and filtered using Whatman filter paper. The filtrate was lyophilized (freeze dried) to powder. The aqueous extract was stoppered in universal bottles and preserved in the refrigerator for use. The extract was thereafter prepared by solubilizing it with distilled water before administration.

2.4 Preparation of Extract for Quantitative Phytochemical Analysis

The fresh leaves of *A. indica* were collected, hand-picked, washed and dried for two weeks at room temperature. It was pulverized to powder using corona manual grinding machine. The dried powdered sample was then used for the quantitative analysis as required by the standard methods used.

2.4.1 Determination of Alkaloids [23].

Five grammes (5g) of the powdered *A. indica* leaf sample was weighed into a 250ml beaker and 200ml of 20% acetic acid in ethanol was added and covered and allowed to stand for 4 hours at 25^{0}C. This was filtered using filter paper no. 42 and the filtrate was concentrated using a water bath (Memmert) to one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitate was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute NH_4OH (1% ammonia solution). Then, filter with pre-weighed filter paper. The residue on the filter paper is the alkaloid, which was dried in the oven (precision electrothermal model BNP 9052 England) at 80^{0}C. The alkaloid content was calculated and expressed as a percentage of the weight of the sample analyzed [23].

Calculation

% weight of alkaloid =

$$\frac{\text{weight of filter paper with residue} - \text{weight of filter paper}}{\text{Weight of sample analyzed}} \times \frac{100}{1}$$

2.4.2 Determination of Saponins [24]

Five grams (5g) of the *A. indica* leaf sample was put into 20% acetic acid in ethanol and allowed to stand in a water bath at 50^{0}C for 24 hours. This was filtered and the extract was concentrated using a water bath to one-quarter of the original volume. Concentrated NH_4OH was added drop-wise to the extract until the precipitate was collected by filtration and weighed. The saponin content was weighed and calculated in percentage using the formula proposed by Obadoni and Ochuko [24].

Calculation

% saponin content =

$$\frac{(\text{weight of filter paper + residue}) - (\text{weight of filter paper})}{\text{Weight of sample analysed}} \times \frac{100}{1}$$

2.4.3 Determination of Tannin by Titration [25]

The Follins Dennis titrating method as described by Pearson (1976) was used. To 20g of the crushed *A. indica* leaf sample in a conical flask was added 100 ml of petroleum ether and covered for 24 hours. The sample was then filtered and allowed to stand for 15 minutes allowing petroleum ether to evaporate. It was then re-extracted by soaking in 100 ml of 10% acetic acid in ethanol for 24 hours. The sample was then filtered and the filtrate collected. Twenty-five ml (25ml) of NH_4OH were added to the filtrate to precipitate the alkaloids. The alkaloids were heated with electric hot plate to remove some of the NH_4OH still in solution. The remaining volume was measured to be 33ml. 5ml of this was taken and 20ml of ethanol was added to it. It was titrated with 0.1M NaOH using phenolphthalein as *indica*tor until a pink end point is reached. Tannin content was then calculated in %($C_1V_1 = C_2V_2$) molarity.

Calculation

$$C_1 = \frac{C_2V_2}{V_1}$$

$$\% \text{ of tannic acid content} = \frac{C_1 \times 100}{\text{Weight of sample analyzed}}$$

C_1 = Conc. of Tannic acid
C_2 = Conc. of base
V_1 = Volume of Tannic acid
V_2 = Volume of Base

2.4.4 Determination of Oxalate by Titration [23]

This determination involves three major steps digestion, oxalate precipitation and permanganate titration.

Digestion

Two (2)g of powdered *A. indica*leaf sample is suspended in 190ml of distilled water in a 250ml volumetric flask. Ten (10) ml of 6M HCl is added and the suspension digested at 100^0C for 1 hour. Cool, and then made up to 250ml mark before filtration.

Oxalate Precipitation

Duplicate portions of 125ml of the filtrate are measured into beakers and four drop of methyl red *indica*tor added. This was followed by the addition of NH_4OH solution (dropwise) until the test solution changes from salmon pink colour to a faint yellow colour (pH4 – 4.5). Each portion was then heated to 90^0c. and 10ml of 5% $CaCl_2$ solution is added while being stirred constantly. After heating, it was cooled and left overnight at 25^0c. The solution is then centrifuged at 2500rpm for 5 minutes. The suspension is decanted and the precipitate completely dissolved in 10ml of 20% (v/v) H_2SO_4 solution.

Permanganate titration

At this point, the total filtration resulting from digestion of 2g of the *A. indica* leaf sample is made up to 300ml. Aliquots of 125ml of the filtrate is heated until near boiling and then titrated against 0.05M standardized $KMnO_4$ solution to a faint pink colour which persists for 30s. The calcium oxalate content is calculated using the formula:

$$\frac{T \times (Vme)(Df) \times 10^5}{(ME) \times Mf} \text{ (mg/100g)}$$

T = Titre of $KMnO_4$(ml),
Vme = Volume-mass equivalent (i.e. 1ml of 0.05m$KMnO_4$ solution is equivalent to 0.00225g anhydrous oxalic acid),
Df = Dilution factor Vt/A (2.4 where Vt is the total volume of titre (300ml) and A is the aliquot used (125ml),
ME = Molar equivalent of $KMnO_4$ in oxalate ($KMnO_4$ redox reaction),
Mf = Mass of sample used.

2.4.5 Determination of Flavonoids [26]

Ten gramme (10g) of the powdered *A. indica* leaf sample was extracted repeatedly with 100ml of 80% methanol at room temperature. The whole solution was filtered through Whatman filter paper No. 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight [26].

Calculation

% Flavonoids =

$$\frac{(\text{weight of crucible + residue}) - (\text{weight of crucible})}{\text{Weight of sample analyzed}} \times \frac{100}{1}$$

2.4.6 Determination of Cardiac Glycosides [27]

To 1ml of *A. indica*leaf extract was added 1ml of 2% solution of 3,5-DNS (Dinitro Salicylic acid) in methanol and 1ml of 5% aqueous NaOH. It was boiled for 2 minutes (until brick-red precipitate was observed) and the boiled sample was filtered. The weight of the filter paper was weighed before filtration. The filter paper with the absorbed residue was dried in an oven at 50^0c till dryness and weight of the filter paper with residue was noted. The cardiac glycoside was calculated in %.

Calculation

% Cardiac glycoside =

$$\frac{(\text{weight of filter paper + residue}) - (\text{weight of filter paper})}{\text{Weight of sample analyzed}} \times \frac{100}{1}$$

2.4.7 Determination of Phytate [28]

Phytate content was determined using the method of Young and Greaves [28] as adopted by Lucas Markakes [29]. Two (2g) of each of the differently prepared powdered neem leaf sample was weighed into different 250ml conical flasks. Each sample was soaked in 100ml of 2% concentrated HCl for 3 hrs. The sample was then filtered. 50ml of each filtrate was laced in 250ml beaker and 100ml distilled water added to each sample. 10ml of 0.3% ammonium thiocynate solution was added as *indica*tor and titrated with standard iron (III) chloride solution which contained 0.00195g iron per 1ml.

$$\text{Phytic acid} = \frac{\text{Titre value} \times 0.00195 \times 1.19}{\text{Weight of sample}} \times \frac{100}{1}$$

2.4.8Determination of Phenol [30]

The quantity of phenols was determined using the spectrophotometer method. The *A. indica* leaf sample was boiled with 50ml of $(CH_3CH_2)_2$ for 15 minutes. Exactly 5ml of the boiled sample was then pipetted into 50ml flask, and 10ml of distilled water was added. After the addition of distilled water, 2ml of NH_4OH solution and 5ml of concentrated $CH_3(CH_2)_3CH_2OH$ was added to the mixture. The sample was made up to the mark and left for 30 mins to react for colour development and measured at 505 nm wavelength using spectrophotometer.

2.4.9 Determination of Steroid Content [30]

One (1.0g) of the powdered *A. indica* leaf sample was weighed and mixed in 100ml of distilled water in a conical flask. The mixture was filtered and the filtrate eluted with 0.1N ammonium hydroxide solution. 2ml of the eluent was put in a test tube and mixed with 2ml of chloroform. Three (3) ml of ice-cold acetic anhydride was added to the mixture in the flask. 2 drops of (200 mg/dl) standard sterol solution was prepared and treated as described for test as blank. The absorbance of standard and test was measured, zeroing the spectrophotometer with blank at 420 nm.

Calculation

$$\text{Mg/100ml)} \ \frac{\text{Absorbance of test} \times \text{Conc of Standard}}{\text{Absorbance of standard}}$$

2.4.10 Determination of Heamaglutinin [31]

To two gramme (2g) of each of the powdered *A. indica*leaf sample were added 20ml of 0.9% NaCl and the suspension shaken vigorously for 1 min. The supernatant was left to stand for 1 hr. The samples were then centrifuged at 2000rpm using centrifuge (PEC MEDICAL USA) for 10 min and the suspension filtered. The supernatants in each were collected and used as crude agglutination extract. The absorbance was readat 420nm.

2.5 Investigation of Antidiabetogenic (Protective) Properties

A total of one hundred (100) rats were randomized intofour (4) groups (n=25) and used for the study. Each group of 25 rats was sub-divided into five (5) groups (n=5) as shown in table 1. Exactly 100mg/kg, 200mg/kg and 400mg/kg body weight of the aqueous leaf extract of *A. indica*leaves were consecutively administered for 7 days, 14 days, 21 days and 28 days before the induction of diabetes. Administration was by oral gavage using intubation cannular. Body weights and blood glucose levels of rats were determined before initiating the daily administration of the extracts. Body weights and blood glucose levels of rats were monitored every two days consistently for the duration of repeated dose before induction of diabetes. Diabetes mellitus was induced intraperitoneally with 50mg/kg b.w. of streptozotocin. The onset of diabetes was established by increase in the normal fasting blood sugar level (between 60mg/dl to 120mg/dl) to above 200mg/dl [32]. After induction with streptozotocin the weights of rats, the blood glucose level as well as other symptoms of diabetes were determined and monitored.

Table 1: Grouping of animals for antidiabetogenic study.

Grouping	Dose per Kg	No. of Rats
Group A (Normal Control)	No pretreatment	5
Group B (Metformin)	100mg	5
Group C (Aqueous extract)	100mg	5
Group D (Aqueous extract)	200mg	5
Group E (Aqueous extract)	400mg	5

2.6 Data Analysis

Data obtained were analyzed using the Statistical Package for Social Sciences (SPSS) software for windows version 25 (SPSS Inc., Chicago, Illinois, USA). All the data were expressed as Mean ± SD. The results obtained were subjected to Analysis of Variance (ANOVA) to determine if significant difference exists between the mean of the test and control groups at $p<0.05$.

3. RESULTS

3.1 Result of Quantitative Phytochemical Analysis

The result of the quantitative phytochemical analysis of the powdered leaves of *A. indica* revealed the quantities of saponin, alkaloids, cardiac glycosides, flavonoids, heamaglutinin, oxalate, phenol, phytate, steroids and tannin in the sample (Table 2). The percentage composition of the phytochemicals in the leaves of neem is as follows: Alkaloids (11.36%),cardiacglycosides(6.19%),flavonoid(5.34%), Heamaglutinin (6.331%), Oxalate (0.0204%), phenol (4.312%), phytate (4.600%), saponin (12.55%), steroid (0.2566) and tannin (6.17%).

Table 2: The mean of the triplicate values of the percentage composition of the phytochemical constituents of *A. indica* leaf.

Phytochemicals	Proximate Composition (%)
Alkaloids	11.36
Cardiac Glycosides	6.150
Flavonoids	5.347
Heamaglutinin	6.331
Oxalate	0.02040
Phenol	4.312
Phytate	4.600
Saponin	12.55
Steroids	0.2598
Tannin	6.153

3.2 Weight following pre-treatment with aqueous *A. indica* leaf extract

The groups that were pre-treated for seven days with 100, 200 and 400mg/kg bw of aqueous extract of *A. indica* before the induction of diabetes showed 8.12%, 6.35% and 4.64% reduction respectively in their weight 48 hours after the induction of diabetes compared to the group pre-treated with metformin (7.25%) and the untreated group (9.89%) (table 3). The result showed that a better protection against weight loss was observed in the group pre-treated with 400mg/kg bw. The groups that were pre-treated for fourteen days with 100, 200 and 400mg/kg bw of aqueous extract of *A. indica* before the induction of diabetes showed 5.68%, 5.56% and 4.18% reduction respectively in their weight 48 hours after the induction of diabetes compared with the group pre-treated with metformin (8.85%) and the untreated group (9.67%) (table 4). The result revealed that at two weeks all the doses tested showed better protection against weight loss.

The groups that were pre-treated for twenty-one days with 100, 200 and 400mg/kg bw of aqueous extract of *A. indica* before the induction of diabetes showed 6.67%, 6.05% and 4.69% reduction respectively in their weight 48 hours after the induction of diabetes compared with the group pre-treated with metformin (8.04%) and the untreated group (9.90%) (table 5). The result showed that a better protection against weight loss was observed in the group pre-treated with 400mg/kg bw. The groups that were pre-treated for twenty-eight days with 100, 200 and 400mg/kg bw of aqueous extract of *A. indica* before the induction of diabetes showed 7.04%, 5.97% and 2.50% reduction respectively in their weight 48 hours after the induction of diabetes compared with the untreated group (10.8) and the group pre-treated with metformin (6.02%) (table 6). The result showed that a better protection against weight loss was observed in the group pre-treated with 400mg/kg bw followed by the group pre-treated with 200mg/kg bw.

Table 3: Weight (g) profile of rats during one-week pre-treatment with 100mg/kg, 200mg/kg and 400mg/kg b.w. of aqueous extract of *A. indica* expressed as mean ± SD**.**

Time (Days)	Untreated Rats	100mg/kg Metformin	100 mg/kg Aqueous Extract	200 mg/kg Aqueous Extract	400 mg/kg Aqueous Extract
Day 0	124.9±2.646	126.6±4.393	126.8±4.919	122.6±3.131	128.4±4.930
Day 2	125.4±2.302	128.0±4.796	128.4±4.827	125.2±2.864	130.2±5.630
Day 4	126.8±2.775	129.6±5.899	129.6±52.53	126.0±2.915	132.4±4.669
Day 6	127.0±2.345	131.8±5.933	132.2±5.069	128.8±2.387	134.2±4.868
Day 7	129.4±2.510	135.2±6.261	135.4±4.615	132.2±2.775	137.8±3.701
Induction of diabetes with streptozotocin after 16 hours fasting					
Day 10	116.6±4.278	125.4±8.142	124.4±4.099	123.8±3.493	131.4±5.771
% decrease in weight	9.89	7.25	8.12	6.35	4.64bd

[a]significant reduction with respect to untreated control; [b]significant increase with respect to untreated control; [c]significant reduction with respect to positive control (metformin); [d]significant increase with respect to positive (metformin) control.

Table 4: Weight (g) profile of rats during two weeks pre-treatment with 100mg/kg, 200mg/kg and 400mg/kg b.w. of aqueous extract of *A. indica*expressed as mean ± SD**.**

Time (Days)	Normal (Untreated) Rats	100mg/kg Metformin	100 mg/kg Aqueous Extract	200 mg/kg Aqueous Extract	400 mg/kg Aqueous Extract
Day 0	124.0±2.646	136.2±3.962	128.6±5.595	124.6±3.362	122.0±3.937
Day 2	125.4±2.302	136.4±4.336	128.8±5.450	124.4±2.793	122.2±3.564
Day 4	126.8±2.775	138.8±7.362	132.2±5.933	126.6±2.881	122.6±4.159
Day 6	127.0±2.345	139.6±3.975	131.2±5.586	127.2±1.483	125.0±4.637
Day 8	131.4±2.702	141.6±6.986	131.0±4.637	131.3±2.872	127.2±3.564
Day 10	132.8±5.450	143.6±5.550	135.5±4.435	131.5±4.655	130.0±3.317
Day 12	133.6±2.608	146.0±7.348	137.0±4.690	134.0±4.690	134.2±4.324
Day 14	134.4±4.669	149.2±6.301	140.8±4.193	138.5±2.517	134.0±4.062
Induction of diabetes with streptozotocin after 16 hours fasting					
Day 17	121.4±3.362	136.0±8.775	132.8±2.500	130.8±3.594	128.4±3.362
% decrease in weight after induction of Diabetes mellitus	9.67	8.85	5.68bd	5.56bd	4.18bd

[a]significant reduction with respect to untreated control; [b]significant increase with respect to untreated control; [c]significant reduction with respect to positive control (metformin); [d]significant increase with respect to positive (metformin) control.

Table 5: Weight (g) profile of rats during three weeks pre-treatment with 100mg/kg, 200mg/kg and 400mg/kg b.w. of aqueous extract of *A. indica*expressed as mean ± SD**.**

Time (Days)	Normal (Untreated) Rats	100mg/kg Metformin	100 mg/kg Aqueous Extract	200 mg/kg Aqueous Extract	400 mg/kg Aqueous Extract
Day 0	124.0±2..646	124.4±2.608	123.0±3.082	123.0±2.739	122.4±2.302
Day 2	125.4±2.302	126.0±1.732	122.6±2.302	124.6±2.509	124.4±1.817
Day 4	126.8±2.775	127.2±2.049	124.8±2.280	126.4±2.408	126.2±1.095
Day 6	127.0±2.345	128.2±2.168	126.6±1.517	119.2±3.194	128.4±1.342
Day 8	131.2±2.775	130.8±1.304	127.6±1.342	125.8±3.421	130.0±1.225
Day 10	131.4±2.702	133.2±2.280	129.0±2.345	128.0±3..317	131.6±2.074
Day 12	133.6±2.608	134.4±0.894	131.8±0.837	130.2±3.633	134.0±2.000
Day 14	132.8±5.450	137.0±1.414	133.6±1.517	132.0±3.742	133.8±3.633
Day 16	134.4±4.669	138.6±1.140	134.6±2.966	134.2±3.633	137.4±2.191
Day 18	136.0±3.464	140.0±2.345	135.5±3.416	136.0±3.317	140.2±1.924
Day 20	137.0±3.082	141.2±1.923	139.0±2.944	138.0±3.317	142.0±2.121
Day 21	139.4±3.361	144.2±1.095	142.5±1.915	142.2±3.701	145.0±2.000
Induction of diabetes with streptozotocin after 16 hours fasting					
Day 24	125.6±4.159	132.6±2.702	133.0±1.414	133.6±3.647	138.2±3.114
% decrease in weightafter induction of DM	9.90	8.04	6.67b	6.05b	4.69bd

[a]significant reduction with respect to untreated control; [b]significant increase with respect to untreated control; [c]significant reduction with respect to positive control (metformin); [d]significant increase with respect to positive (metformin) control.

Table 6: Weight (g) profile of rats during four weeks pre-treatment with 100mg/kg, 200mg/kg and 400mg/kg b.w. of aqueous extract of *A. indica* expressed as mean ± SD**.**

Time (Days)	Normal (Untreated) Rats	100mg/kg Metformin	100 mg/kg Aqueous Extract	200 mg/kg Aqueous Extract	400mg/kg Aqueous Extract
Day 0	124.0±2.646	124.2±4.087	121.6±2.302	126.6±4.669	125.6±4.219
Day 2	126.8±2.775	127.0±3.391	123.2±1.304	130.2±4.207	129.4±3.847
Day 4	127.0±2.345	128.8±5.675	125.0±1.581	131.8±4.658	131.4±3.847
Day 6	129.4±2.510	129.4±4.722	126.8±1.789	133.4±4.506	133.4±3.847
Day 8	131.4±2.702	131.6±3.209	127.8±2.387	135.0±4.301	135.0±3.162
Day 10	133.6±2.608	134.2±4.087	129.6±1.949	136.8±4.147	136.6±2.881
Day 12	132.8±5.450	136.4±3.578	131.8±2.500	139.0±4.183	138.4±2.966

Day 14	136.0±3.464	137.8±3.564	133.0±0.817	140.0±4.183	140.0±3.391
Day 16	137.0±3.082	139.4±4.278	135.0±0.000	141.8±3.834	141.8±3.421
Day 18	139.4±4.670	142.4±4.278	137.0±0.817	143.6±3.975	143.4±3.362
Day 20	139.4±3.362	144.6±4.506	139.5±0.577	145.6±3.975	144.8±2.382
Day 22	141.6±3.578	145.8±5.167	141.3±1.258	147.8±3.633	147.5±2.380
Day 24	142.8±2.683	150.0±6.000	143.0±1.000	149.8±3.633	149.3±2.217
Day 26	145.0±4.301	148.6±3.782	144.7±0.577	151.8±3.564	151.0±1.826
Day 28	147.6±2.966	152.8±3.493	147.7±1.155	154.0±3.317	152.3±2.217
Induction of diabetes with streptozotocin after 16 hours fasting					
Day 31	131.6±2.408	143.6±4.159	137.3±2.517	144.8±5.541	148.5±1.732
% decrease in weight after induction of DM	10.8	6.02	7.04b	5.97b	2.50b

[a]significant reduction with respect to untreated control; [b]significant increase with respect to untreated control; [c]significant reduction with respect to positive control (metformin); [d]significant increase with respect to positive (metformin) control.

3.3 Glucose Levels following pre-treatment with *A. indica* leaf extract

The result of the daily fasting blood glucose levels of the different group of rats administered 100mg/kg, 200mg/kg and 400mg/kg bw. of *A. indica* leaf extract for 7, 14, 21 and 28 days before the induction of diabetes were shown in tables 7, 8, 9 and 10 respectively. Also, the fasting blood glucose levels of the rats were recorded 48 hrs after the induction of diabetes. The difference in percentage increase in blood glucose levels between the normal untreated rats and those administered extracts at all dose levels remain about the same after 7 days and shows marked difference from 14 days and increases($p<0.05$) significantly as the duration of pre-treatment increases.

There was no significant ($p<0.05$) difference in the fasting blood glucose levels for the group of animals pre-treated for seven days with the aqueous leaf extracts of *A. indica* compared with the untreated group after the induction of diabetes (table 7). The result of the daily fasting blood glucose levels of the different group of rats administered graded doses of *A. indica* extract for fourteen (14) days before the induction of diabetes was shown in table 8. The groups that were pre-treated with the aqueous extract of *A. indica* leaf extracts for fourteen days showed a significant ($p<0.05$) reduction in the fasting blood glucose level after the induction of diabetes compared with the normal rats that were not pre-treated (table 8). There was a significant ($p<0.05$) difference in the percentage increase in fasting blood glucose level of the groups pre-treated with the graded doses of the extract compared with the normal untreated group and the group pre-treated with a standard antidiabetic drug (metformin).

The result of the daily fasting blood glucose levels of the different group of rats administered 100mg/kg, 200mg/kg and 400mg/kg bw. of aqueous extract of *A. indica* for twenty-one (21) days before the induction of diabetes was shown in table 9. The groups that were pre-treated for twenty-one days with aqueous extract of *A. indica* leaf before the induction of diabetes showed a significant ($p<0.05$) decrease in their fasting blood glucose levels after the induction of diabetes compared with the normal untreated rats and the rats pre-treated with a standard antidiabetic drug (table 9). The result of the daily fasting blood glucose levels of the different group of rats administered graded doses of *A. indica* leaf extract for twenty-eight (28) days before the induction of diabetes was shown in table 10. The groups that were pretreated for twenty-eight days with aqueous extract of *A. indica* leaf before the induction of diabetes showed a significant ($p<0.05$) decrease in their fasting blood glucose levels after the induction of diabetes compared with the normal untreated rats (table 10).

Table 7: Profile of fasting blood glucose (mg/dl) levels during one-week pre-treatment with 100mg/kg, 200mg/kg and 400mg/kg b.w. of aqueous extract of *A. indica* leaf expressed as mean ± SD**.**

Time (Days)	Normal (Untreated)	100mg/kg Metformin	100 mg/kg Aqueous Extract	200 mg/kg Aqueous Extract	400 mg/kg Aqueous Extract
Day 0	96.00±12.75	97.60±19.07	100.6±15.34	79.60±11.91	93.20±19.93
Day 2	97.80±10.33	95.80±14.45	99.20±22.38	83.20±7.530	96.60±15.85
Day 4	98.20±9.731	94.60±15.93	103.8±12.70	85.00±3.162	92.00±17.76
Day 6	95.00±10.56	98.60±18.58	95.4±19.97	90.00±9.747	90.40±15.27
Day 7	99.00±7.382	96.00±18.72	104.8±10.76	84.20±12.00	91.40±11.82
Induction of diabetes with streptozotocin after 16 hours fasting					
Day 10	538.8±4.592	533.4±94.76	499.0±82.91	554.2±46.99	480.8±96.42
% increase in glucose level after induction of DM	81.6	82	78.9	84.4	81.0

[a]significant reduction with respect to untreated control; [b]significant increase with respect to untreated control; [c]significant reduction with respect to positive control (metformin); [d]significant increase with respect to positive (metformin) control.

Table 8: Profile of glucose (mg/dl) levels during two weeks pre-treatment with 100mg/kg, 200mg/kg and 400mg/kg b.w. of aqueous extract of *A. indica*leaf expressed as mean ± SD**.**

Time (Days)	Normal (Untreated)	100mg/kg Metformin	100 mg/kg Aqueous Extract	200 mg/kg Aqueous Extract	400 mg/kg Aqueous Extract
Day 0	96.00±12.75	91.80±13.93	95.20±8.289	101.4±11.61	91.20±9.576
Day 2	97.80±10.33	88.40±14.21	95.80±9.628	97.00±11. 40	93.40±8.820
Day 4	98.20±9.731	85.00±7.969	94.20±8.526	94.80±11.58	92.20±12.87
Day 6	95.00±10.56	88.20±9.418	99.00±9.301	90.20±7..040	95.40±12.50
Day 8	99.00±7.382	92.20±9.338	94.00±5.523	92.20±5.263	101.6±11.13
Day 10	96.20±8.786	86.80±3.701	91.25±4.787	88.25±2.986	93.20±16.33
Day 12	100.0±2.382	89.20±4.712	95.50±5.260	91.25±4.646	96.80±17.70
Day 14	94.40±10.92	91.00±10.07	96.00±4.967	91.00±5.598	98.20±10.52
Induction of diabetes with streptozotocin after 16 hours fasting					
Day 17	577.0±23.19	544.8±88.20	472.8±175.8	487.8±75.57	457.2±98.74
% increase after induction	83.6	83.3	79.7	81.3	78.4

[a]significant reduction with respect to untreated control; [b]significant increase with respect to untreated control; [c]significant reduction with respect to positive control (metformin); [d]significant increase with respect to positive (metformin) control.

Table 9: Profile of glucose (mg/dl) levels during three weeks pre-treatment with 100mg/kg, 200mg/kg and 400mg/kg b.w. of aqueous extract of *A. indica* leaf expressed as mean ± SD**.**

Time (Days)	Normal (Untreated)	100mg/kg Metformin	100 mg/kg Aqueous Extract	200 mg/kg Aqueous Extract	400 mg/kg Aqueous Extract
Day 0	96.00±12.75	93.60±9.839	108.6±9.476	98.80±9.257	97.00±9.192
Day 2	97.80±10.33	92.00±9.618	109.0±5.788	96.00±7.616	95.20±6.017
Day 4	98.20±9.731	92.00±7.810	103.2±8.228	96.00±9.000	95.40±9.711
Day 6	95.00±10.56	92.40±6.348	103.0±11.29	92.20±5.070	92.40±10.95
Day 8	99.00±7.382	95.80±3.768	95.00±3.873	96.80±8.136	95.60±9.889
Day 10	100.0±2.345	93.00±5.612	93.20±5.310	99.00±11.31	95.80±14.46
Day 12	96.20±8.786	88.80±4.817	89.60±12.60	98.20±22.56	87.80±8.927
Day 14	94.40±10.92	91.60±5.857	95.40±10.29	93.00±6.205	86.00±6.782
Day 16	94.80±7.155	91.20±6.496	96.00±12.41	98.60±12.12	83.80±7.530
Day 18	90.40±9.236	92.80±7.791	90.25±11.95	91.20±7.662	85.20±10.11
Day 20	98.60±5.683	87.20±8.526	94.50±10.54	87.80±6.221	84.40±3.715
Day 21	94.40±11.06	89.88±8.871	107.3±8.421	93.40±8.142	87.80±6.140
Induction of diabetes with streptozotocin after 16 hours fasting					
Day 24	545.4±88.60	580.0±23.84	310.5±42.24	390.8±95.06	317.8±49.61
% increase in glucose level after induction of DM	82.7	84.5	65.4bd	76.3bd	72.4bd

[a]significant reduction with respect to untreated control; [b]significant increase with respect to untreated control; [c]significant reduction with respect to positive control (metformin); [d]significant increase with respect to positive (metformin) control.

Table 10: Profile of glucose (mg/dl) levels during four weeks pre-treatment with 100mg/kg, 200mg/kg and 400mg/kg b.w. of aqueous extract of *A. indica* leaf expressed as mean ± SD**.**

Time (Days)	Normal (Untreated)	100mg/kg Metformin	100 mg/kg Aqueous Extract	200 mg/kg Aqueous Extract	400 mg/kg Aqueous Extract
Day 0	96.00±12.75	82.40±12.14	87.40±9.017	98.40±10.83	108.6±8.204
Day 2	98.20±9.731	83.60±5.595	87.40±6.148	94.20±11.73	106.0±7.382
Day 4	95.00±10.560	85.60±7.668	86.60±6.841	95.60±13.85	86.80±7.918
Day 6	100.0±2.345	82.20±7.014	90.40±6.229	86.80±10.55	87.60±7.537
Day 8	99.00±7.382	81.00±5.874	88.00±5.701	86.40±9.633	86.00±7.416
Day 10	96.20±8.786	82.00±11.60	90.20±11.37	89.00±11.68	90.00±5.568
Day 12	94.80±7.155	84.80±9.757	83.75±9.570	85.60±4.980	89.00±7.246
Day 14	94.40±10.92	86.20±8.758	88.75±6.238	95.20±10.12	95.40±11.46

Day 16	90.40±9.236	81.40±9.711	86.25±5.252	91.20±11.21	89.00±7.246
Day 18	98.60±5.683	85.60±8.989	75.75±4.272	94.40±12.18	98.80±11.14
Day 20	94.40±11.06	83.80±5.975	82.50±4.796	100.6±6.731	88.60±4.561
Day 22	95.80±10.73	84.40±8.532	82.00±3.606	95.60±9.555	94.40±11.35
Day 24	89.00±13.80	82.60±5.413	87.34±2.631	97.60±9.072	73.60±29.13
Day 26	93.60±10.19	85.60±4.393	85.67±6.429	97.60±10.99	80.20±35.27
Day 28	95.20±12.46	83.60±4.099	86.67±4.726	93.40±11.46	98.00±15.38
Induction of diabetes with streptozotocin after 16 hours fasting					
Day 31	569.6±28.76	576.6±37.57	327.0±42.04	296.8±75.67	275.3±60.84
% increase after induction of DM	83.3	85.5	73.5bd	68.5bd	64.4bd

[a]significant reduction with respect to untreated control; [b]significant increase with respect to untreated control; [c]significant reduction with respect to positive control (metformin); [d]significant increase with respect to positive (metformin) control.

4. DISCUSSION

The percentage composition of the phytochemicals in the leaves of *A. indica* was as follows: Alkaloids (11.36%), cardiac glycosides (6.19%), flavonoid (5.34%), Heamaglutinin (6.331%), Oxalate (0.0204%), phenol (4.312%), phytate (4.600%), saponin (12.55%), steroid (0.257%) and tannin (6.17%). A qualitative phytochemical analysis conducted on the Aqueous, Ethanol, and Chloroform extracts of the neem leave by Ramadas and Subramanian [33], revealed that the ethanol extract of *A. indica* has alkaloids, glycosides, flavonoids, saponins, tannins, phenolic compounds and reducing sugar. A Study conducted by Koona and Budida [34], revealed that triterpenoids, phenolic compounds,carotenoids, steroids, valavinoids, ketones and tetra-triterpenoids,azadirachtinobserved in *Azadirachta indica*might be responsible for the antibacterial activity of the leave which is responsible for their uses as herbs in primary health care. These phytochemicals are beneficial to the plants as they serve as important metabolites that help the plant to exhibit its pharmacological role in treatment and management of diseases. The phytochemicals analyzed in the leaves of *A. indica* may be responsible for its antidiabetogenic property.

The percentage change in weight of the rats after the induction of diabetes compared with the normal untreated rats and the rats pre-treated with a standard antidiabetic drug decrease significantly ($p<0.05$)after twenty-eight days with respect to the graded doses of the *A. indica* extract (100mg/kg, 200mg/kg and 400mg/kg b. w of *A. indica* leaf extract). *A. indica* extracts Pretreatment with extract of *A. indica* leaf hinders weight loss and this is confirmed by the findings of Chattopadhay and Bandyopadhyay [35]. Gupta *et al*. [36] revealed that controlled blood glucose level lowers the breakdown of structural protein leading to the decrease in weight loss.

The groups that were pretreated for twenty-one days with aqueous extract of *A. indica* leaf before the induction of diabetes showed a significant ($p<0.05$) decrease in their fasting blood glucose levels after the induction of diabetes compared with the normal untreated rats and the rats pre-treated with a standard antidiabetic drug(table 9). This result is in agreement with the findings of Ezeigwe*et al*. [22] who reported the controlling effect of *A. indica* leaf extract on blood glucose levels thereby delaying the onset of diabetes.

The groups that were pretreated for twenty-eight days with graded doses of aqueous extracts of *A. indica* leaf before the induction of diabetes showed a significant ($p<0.05$) decrease in their fasting blood glucose levels after the induction of diabetes compared with the normal untreated rats (table 10). These results suggest that the longer the administration of the extract the more its protective effects against the onset of diabetes, which confirms the result reported byOkpe*et al*. [37]. Jelodar*et al*. [21] suggested that the antidiabetic properties of neem extract is as a result of its ability to quicken the sufficient production of insulin by the pancreas which utilize glucose in the cell or possibly, the competence of the extract to regenerate the beta cell to carry out its functions. In a research work byGupta *et al*. [38], it was stated that the extract shows significant protection against the oxidative damage induced by STZ in heart and erythrocytes of rats.

5. CONCLUSION

The longer the period of pre-treatment with the aqueous extract of *A. indica* leaves the better the protection against the onset of diabetes. This entails that administration of the extract can delay the onset of diabetes in diabetes prone subjects. This could be by quickening the sufficient production of insulin by the pancreas which activates the glucose transporters to transport glucose to the cells for effective utilization or possibly, the capability of the extract to regenerate the beta cells to produce enough insulin needed to signal the glucose transporters to carry glucose to the cells.

Ethical Approval

The authors hereby declare that “Principles of Laboratory Animal Care” were followed. The experiments were examined and approved by the ethics committee of Nnamdi Azikiwe University, Awka, Nigeria in accordance with the Institutional Animal Care and Use policy in Research, Education and Testing.

Competing Interests

The authors state no conflict of interest in this research.

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Authors Contribution

Authors OCE and FCE designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Authors OCE and NNN managed the literature searches and the analysis of the study. All authors read and approved the final manuscript.

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