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Phytochemical Constituents and Antidiabetic Property of *Cola nitida* Seeds on Alloxan-Induced Diabetes Mellitus in Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author SOO designed the study and manage the laboratory analysis of the study. Author IUD managed the literature searches, interpreted the data and wrote the first draft of the manuscript, while authors EED and LLM performed the statistical analysis and managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

The seeds of *Cola nitida* were investigated for their anti-diabetic properties. The extracts were obtained by maceration in cold water. Alloxan was used to induce diabetes mellitus (DM) in the rats at 180mg/kg body weight and diabetes was confirmed after 48 hours. The Phytochemical analyses of the plants showed the presence of tannin, flavonoid, terpenoid, cardiac-glycoside, and alkaloids. Anthraquinone and saponin were observed to be absent. *Cola nitida* was also observed to cause a reduction in blood glucose level from 599±0.667mg/dl (diabetic control) to 170±0.577mg/dl (extract treated group). The extract showed a significant ($P<0.05$) decrease of blood glucose level in alloxan induced diabetic rats. *Cola nitida* aqueous extract showed a higher level of potency when compared to a known anti-diabetic drug (Glanil).

Keywords: Antidiabetic; diabetes mellitus; phytochemical; *Cola nitida*.

1. INTRODUCTION

Diabetes mellitus is a condition in which the body does not produce enough, or properly respond to insulin, a hormone produced in the pancreas. This causes hyperglycemia, often leading to various complications. Insulin stimulates the uptake of glucose for energy [1,2]. Type 1 diabetes mellitus results from the body's failure to produce insulin. Type 2 diabetes mellitus which is the more prevalent form may have as its underlying metabolic causes the combined effects of impairment in the insulin mediated glucose disposal and defective secretion of insulin by the β -cells of the pancreas [3].

Diabetes mellitus has been conventionally treated with orthodox medicines that function as hypoglycaemic agents, or insulin production modulators and/or lipoprotein lowering agents [4]. Sulfonylurea and metformin are valuable in the treatment for hyperglycaemia in type 2 DM but they are often unable to lower glucose concentrations to within the normal range, or to reinstate a normal pattern of glucose homeostasis [5]. Even when effective glycaemic control is achieved, the use of these drugs is restricted by their pharmacokinetic properties, secondary failure rates and accompanied undesirable effects [6,7]. Acute complications including hypoglycemia, diabetic ketoacidosis, or nonketotic hyperosmolar coma may occur if the disease is not adequately controlled. Serious long-term complications include cardiovascular disease, chronic renal failure, retinal damage, which can lead to blindness, several types of nerve damage, and microvascular damage, which may cause erectile dysfunction and poor wound healing. Poor healing of wounds, particularly of the feet, can lead to gangrene, and possibly to amputation [8]. Adequate treatment of diabetes mellitus, as well as increased emphasis on blood pressure control and lifestyle factors such as not smoking and maintaining a healthy body weight, may improve the risk profile of most of the chronic complications. In the developed world, diabetes mellitus is the most significant cause of adult blindness in the non-elderly and the leading cause of non-traumatic amputation in adults, and diabetic nephropathy is the main illness requiring renal dialysis [8].

Most of the orthodox medicines used in treatment of diabetes mellitus are not suitable for use during pregnancy [5,9]. Since the therapy is life long, therapeutic agents devoid of side effects would be appreciated and one of such approach is the use of alternative system of medicine comprising herbal products [10]. For these reasons, therefore, there is a great need for a search of an acceptable, cheap and safe blood glucose lowering agents that

would be effective in the treatment of diabetes mellitus and devoid of serious side effects of the currently used oral hypoglycaemic agents. *Cola nitida* has been suspected to have hypoglycemic effects though it has not been an area of extensive research [11].

Cola nitida is a genus of about 125 species of trees native to the tropical rainforests of Africa, classified in the family *Malvaceae*, subfamily *Sterculioideae*. They are evergreen trees, growing to 20 m tall, with glossy ovoid leaves up to 30 cm long [12]. It is valued for its caffeine content and stimulant properties. It is also useful for fighting fatigue and stimulating the central nervous system. It contains theobromine, the same stimulant found in green tea [13]. The American Medical Association recommends cola nut over other types of stimulants because it is non-habit-forming and does not cause depression [12]. It is also a known diuretic and therefore may help with renal and rheumatic conditions. Cola nut helps expand bronchial air passages and therefore is useful for treating conditions like asthma and whooping cough. It also acts as an appetite suppressant and stimulates digestion by activating the production of gastric juices [14].

Due to the information by local herbal healers, this present work therefore, aimed at determining the anti-diabetic property of aqueous extract of *Cola nitida* seeds on alloxan-induced diabetes mellitus in rats with a view to certify the claim by local herbal healers for the seed's potency or otherwise.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Material

Cola nitida seeds were purchased from Akure, Ondo state of Nigeria and were identified by Mrs. Jemilat Ibrahim a botanist of the Herbarium Unit, National Pharmaceutical Institute of Research and Development (NIPRD) Idu, Abuja Nigeria where voucher number 22099 was assigned.

2.2 Extraction of Plant Material

The seeds were chopped into smaller pieces, dried at room temperature (37°C) for four weeks, and then ground to fine powder, using a blender. The powdered material (20g) was percolated with 100ml of water. It was then filtered and concentrated in a rotary evaporator. The extract obtained was weighed (4.7g) and stored in the refrigerator until when needed.

2.3 Phytochemical Screening

Phytochemical tests were carried out on the aqueous extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara; Trease and Evans [15,16].

2.4 Test for Tannins

Briefly, 0.5 g of the dried powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration which indicates the presence of tannins.

2.5 Test for Saponin

Briefly, 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously, then observed for the formation of emulsion.

2.6 Test for Flavonoids

Briefly, three methods were used to determine the presence of flavonoids in the plant sample [14]. 5 ml of dilute ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated sulfuric acid. A yellow colouration observed in each extract indicated the presence of flavonoids. The yellow colouration disappeared on standing. Few drops of 1% aluminium solution were added to a portion of each filtrate. A yellow colouration was observed indicating the presence of flavonoids. A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colouration was observed indicating a positive test for flavonoids.

2.7 Test for Steroids

Briefly, 2 ml of acetic anhydride was added to 0.5 g ethanolic extract of each sample with 2 ml sulfuric acid. The colour changed from violet to blue or green in some samples indicating the presence of steroids.

2.8 Test for Terpenoids (Salkowski Test)

Exactly 5 ml of each extract was mixed in 2 ml of chloroform, and concentrated sulfuric acid (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface was formed to show positive results for the presence of terpenoids.

2.9 Test for Cardiac Glycosides (Keller-Killani Test)

Briefly, 5ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was underlayered with 1 ml of concentrated sulfuric acid. A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

2.10 Test for Anthraquinones

Briefly, 0.5g of the extract was boiled with 10ml of sulfuric acid and filtered while hot. The filtrate was shaken with 5ml of chloroform. The chloroform layer was pipette into another test tube and 1 ml of dilute ammonia was added. The resulting solution was observed for colour change (pink, violet or red colour).

2.11 Quantitative Determination of the Phytochemical Constituents

2.11.1 Preparation of fat free sample

Briefly, 2 g of the sample were defatted with 100 ml of diethyl ether using a soxhlet apparatus for 2 hrs.

2.11.2 Determination of total phenols

This was done according to the Folin and Ciocalteu's method as described by Ayoola et al. [17]. The fat free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 min. 5 ml of the extract was pipetted into a 50 ml flask, then 10 ml of distilled water was added. 2 ml of ammonium hydroxide solution and 5 ml of concentrated amylalcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. This was measured at 505 nm.

2.11.3 Alkaloid determination

Alkaloid was determined using the method of Harborne [18]. Briefly, 5 g of the sample was weighed into a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added dropwise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and weighed.

2.11.4 Tannin determination

This was done according to Markkar and Goodchild [19]. Briefly, 500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtrate into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M FeCl_3 in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured at 120 nm within 10 min.

2.11.5 Saponin determination

The method used was that of Obadoni and Ochuko [20]. The samples were ground and 20 g of each were put into a conical flask and 100 cm³ of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4h with continuous stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a waterbath. After evaporation the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage.

2.11.6 Flavonoid determination

Flavonoid content was determined as described by Pham et al. [21]. Exactly, 10 g of the plant sample was extracted repeatedly with 100 ml of 80% aqueous 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.

2.11.7 Experimental animals

Thirty (30) albino rats of both sexes (7-8 weeks old, weighing about 135-150 g) were purchased from the animal house of University of Jos, Nigeria. The animals were allowed to acclimatize for two weeks prior to commencement of the experiment. They were kept at room temperature and were maintained *ad libitum* on tap water and growers mash (Vita feeds, Jos, Plateau State Nigeria). They were weighed prior to commencement and termination of the experiment.

2.11.8 Induction of diabetes and experimental grouping

The rats were randomly divided into six groups of five rats each. Group A, served as the normal control fed with the standard animal feed and water only. Group B, C, D, E and F were administered freshly prepared alloxan monohydrate solution dissolved in normal saline (120mg/kg) intraperitoneally. Blood sample from experimental rats were collected from the tail vein after over-night fasting and hyperglycemia was confirmed forty-eight (48) hours after induction by quantitative determination of blood glucose levels as described by Bowman and Rand [22], using one touch basic glucose monitoring system. Thereafter, group B received no treatment as it was the diabetic control. Group C was treated with Glanil (standard diabetes mellitus drug, 1mg/kg) while groups D, E and F were treated with aqueous extract of *Cola nitida* seed (40, 80 and 120 mg/kg respectively) for fourteen (14) days. Animals in all the groups were sacrificed after light chloroform anesthesia and whole blood was collected and allowed to clot before it was centrifuged to obtain serum. All serum samples were kept in eppendorf tubes and stored at -20°C for biochemical analysis. The study was carried out according to the specifications of the Bingham University Animal Ethical Research Committee.

3. DETERMINATION OF BLOOD PARAMETERS

3.1 Determination of Serum Cholesterol

Cholesterol was determined spectrophotometrically at 570nm using assay kit (Randox Laboratories Ltd, UK) following enzymatic hydrolysis and oxidation as described by Witte et al. [23]. Three test tubes were labeled test, standard and blank. To each of these test tubes was added 0.2ml of serum, cholesterol standard and distilled water respectively. Thereafter, 5.0ml acetic acid-acetic anhydride reagent was added to each of the test tubes and the solution were thoroughly mixed, and then allowed to stand for a period of 5 minutes. 0,1ml of concentrated sulfuric acid was added alongside to each of the tube, mixed thoroughly and kept in cold water for a period of 10 minutes. The absorbance was then read against the blank. Concentration of the test sample was calculated as follows:

$$\text{Concentration of test} = \frac{A (\text{test}) \times \text{Conc. (std)}}{A (\text{std})}$$

3.2 Determination of Liver Function Parameters

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined colorimetrically at 540nm using Randox assay kits based on the principle first described by Reitman and Frankel [24].

Also, using the Randox kit, the colorimetric assay method for conjugated bilirubin employed involved reaction with diazotized sulphanilic acid in alkaline medium to form a blue complex, while total bilirubin was determined in the presence of caffeine, which releases albumin-bound bilirubin that then reacts with diazotized sulphanilic acid as described by Jendrassik and Grof [25].

3.3 Statistical Analysis

Data were expressed as mean \pm SEM. The data obtained were analyzed using one-way analysis of variance (ANOVA). Tukey's multiple comparison post-hoc test was used to determine the level of significance between control and experimental groups. Values of $P < 0.05$ were considered as significant.

4. RESULTS

The results of the phytochemical analysis of *Cola nitida* seeds extract show the presence of some phytochemical compounds and the quantities available as listed in Table 1.

Table 1. Qualitative and quantitative analysis of the phytochemical constituents of *Cola nitida* seed

Phytochemicals	Qualitative result	Percentage of phytochemicals
Tannin	+	0.34 \pm 0.10
Alkaloids	+	0.20 \pm 0.20
Flavonoid	+	0.92 \pm 0.22
Cardiac glycosides	+	10.72 \pm 0.60
Terpenoids	+	11.85 \pm 0.31
Saponin	-	
Anthraquinones	-	

The result of the blood glucose concentration shows significant ($P < 0.05$) reduction in the groups treated with the extract when compared with the diabetic control group and the group treated with the standard drug as shown in Table 2.

From Table 3, after 14 days of treatment with the aqueous extracts of *Cola nitida*, the serum levels of cholesterol significantly ($P < 0.05$) reduced.

Effects of aqueous extract of *Cola nitida* on serum total bilirubin levels after 14 days of treatment is presented in Table 4 below. There was a significant ($P < 0.05$) reduction in the serum level of total bilirubin.

Table 2. Effects of extracts of *Cola nitida* on blood glucose concentration in alloxan-induced diabetic rats (mg/dl)

Treatment	Day 1	Day 5	Day 10	Day 14
Normal (No treatment)	120±0.882	119±0.577	123±0.882	120±1.202
Diabetic control	599±0.667	463±0.882	483±1.856	489±2.848
Glanil control	443±0.577	486±1.202	467±2.082	379±0.882
<i>Cola nitida</i> 40mg/kg of extract	205±1.528*	282±0.577*	154±0.577*	109±0.882*
<i>Cola nitida</i> 80mg/kg of extract	189±0.577*	173±0.882*	156±0.667*	101±1.856*
<i>Cola nitida</i> 120mg/kg of extract	170±0.577*	172±0.577*	157±1.155*	92±0.882*

Mean ± SEM of five determinations. * represents $P < 0.05$ significantly different from diabetic control

Table 3. Effects of aqueous extracts of *Cola nitida* on cholesterol level in alloxan-induced diabetic rats

Treatment	Day14 (mg/dl)
Normal (No treatment)	99.15±0.349
Diabetic control	121.31±26.135
Glanil control	101.21±0.270
<i>Cola nitida</i> 40mg/kg of extract	109.62±0.338
<i>Cola nitida</i> 80mg/kg of extract	84.62±0.445*
<i>Cola nitida</i> 120mg/kg of extract	73.56±0.248*

Mean ± SEM of five determinations. * represents $P < 0.05$ significantly different from diabetic control

Table 4. Effects of *Cola nitida* on serum total bilirubin levels in alloxan-induced diabetic rats

Treatment	Day 14 ($\mu\text{g}/100\text{ml}$)
Normal (No treatment)	5.520±0.001
Diabetic control	9.180±0.010
Glanil control	8.724±0.001
<i>Cola nitida</i> 40mg/kg of extract	6.240±0.002
<i>Cola nitida</i> 80mg/kg of extract	6.068±0.016*
<i>Cola nitida</i> 120mg/kg of extract	5.248±0.001*

Mean ± SEM of five determinations. * represents $P < 0.05$ significantly different from diabetic control

Effects of aqueous extracts of *Cola nitida* on serum enzyme activity {alanine aminotransferase (ALT) and aspartate aminotransferase (AST)} in alloxan induced diabetic rats after 14 days of treatment are presented in Table 5 below. There was decrease in the ALT and AST level in the group treated with 120mg/kg of the extract.

Table 5. Effects of extracts of *Cola nitida* ALT and AST activities in alloxan induced-diabetic rats

Treatment	Day 14 enzyme activity	
	ALT (IU)	AST (IU)
Normal (No treatment)	16.00±0.04	29.00±0.02
Diabetic control	31.00±0.01	43.00±0.01
Glanil control	31.00±0.03	41.00±0.02
<i>Cola nitida</i> 40mg/kg of extract	16.00±0.01	29.00±0.03
<i>Cola nitida</i> 80mg/kg of extract	13.00±0.01*	29.00±0.01
<i>Cola nitida</i> 120mg/kg of extract	10.00±0.03*	25.00±0.02*

Mean ± SEM of five determinations. * represents $P < 0.05$ significantly different from diabetic control

5. DISCUSSION

As a result of the side effects of orthodox drugs used in treatment of diabetes mellitus, attention has been focused on the use of plants and herbal remedies believed to be safe and devoid of serious side effects as alternatives in the treatment of diabetes mellitus. The potency of the aqueous extract of *Cola nitida* was measured in terms of reduction in blood glucose level. There was a significant decrease in blood glucose levels, after 14 days of oral administration of the aqueous extracts of *Cola nitida* in the alloxan-induced diabetic rats. In Comparison with a known anti-diabetic drug Glanil, *Cola nitida* showed a reduction in blood glucose of 26.04% after 24hrs of treatment as shown in Table 2. The major goal in treating diabetes mellitus is controlling elevated blood glucose without causing abnormally low levels of blood glucose [26]. The results in this study are in partial agreement with Mooradian previous study [26]. It can be observed that the blood glucose level of the extract treated group at a dose of 120mg/kg was at the end of the 14days of the experiment showed antidiabetic properties (Table 2). *Cola nitida* has antihyperglycemic properties depending on the administered dosages.

The extracts showed to be effective in decreasing blood glucose levels on the diabetic rats, which could be important to prevent the long-term complications associated with hyperglycemia in diabetes mellitus [4]. The effective lowering of the high blood glucose levels demonstrated by *Cola nitida* extract supports its local use as an antihyperglycemic agent. From Table 3, the extracts significantly reduced the cholesterol levels in the groups treated with extract. Alloxan-induced diabetes mellitus in rats caused a significant increase in the serum cholesterol levels as was observed in the diabetic control rats. This is in accordance with studies carried out in streptozotocin treated animals by Nakayama and Nagakawa [27].

The hepatic and cardiac tissues release AST and ALT and the elevation of serum concentrations of these enzymes is an indicator of hepatic and cardiac damage [28]. The decrease in ALT and AST levels in the diabetic animals treated with different doses of the extract (Table 5) implied that the extract at the doses used did not produce any harmful effects on either the cardiac or the hepatic tissues and also, have protected or ameliorated the cell membrane from the harmful effect of alloxan. However, in the untreated diabetic rats, the two enzymes showed significant increase which suggested that hepatic and cardiac problems may have occurred (Table 5).

There was a significant increase in total bilirubin concentration in the diabetic control rats, following induction of alloxan after 48hrs. However, administration of *Cola nitida* aqueous extracts produced a decrease in bilirubin concentration after 14 days of treatment. The observed elevated bilirubin in the serum of the alloxan-induced diabetic rats in this study could have been due to suppression of bilirubin glucuronidation. The fact that the extract caused a reduction in the serum level of bilirubin as observed in this study suggests that the extract has the potential to protect the red blood cells from damage.

6. CONCLUSION

The study showed that aqueous extracts of *Cola nitida* significantly reduced the elevated levels of blood glucose, cholesterol, bilirubin, AST and ALT in diabetic treated rats. The study also revealed that the extract at doses investigated did not provoke toxic effects to the animals. Due to the above mentioned roles, *Cola nitida* possess antidiabetic property.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (The specifications of the Bingham University Animal Ethical Research Committee) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee of the University.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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