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Glycaemic Activity of the Aqueous Pod Extract of *Acacia nilotica* (Fabaceae) in Normoglycemic and Alloxan Induced Diabetic Wistar Strain Albino Rats

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The study to evaluate glycaemic activity of aqueous pod extract of *Acacia nilotica* in normal and diabetic Wistar albino rats was conducted. Three hundred and fifty grams of the powdered sample were exhaustively extracted with distilled water using reflux method. Phytochemical investigation of the aqueous extract showed that carbohydrate, tannins, saponins, glycosides and flavonoids were present in the extract. The concentrations of Fe, K and Mn in the *Acacia nilotica* pod extract were within safety limit. However, the concentrations of some other elements such as Mn, Zn, Ca, Na, Cd and Cu were much lower than the acceptable levels. Elements like Pb, As and Mo were absent. There was significant ($p<0.05$) decrease by 13.19, 13.95, 11.73, 16.40, 28.80 and 25.46%, respectively in blood glucose level of normal rats and 14.12, 32.28, 49.67, 38.97 and 74.61% in diabetic rats treated with 400, 600 and 800 mg kg^{-1} of aqueous extract at 12 and 18 h post administration. Conclusively it has been deduced from the *in vivo* study that the extract of this plant can be used in the management of hyperglycemic condition, hence can assist in the management of disease condition such as diabetes.

Key words: *Acacia nilotica*, phytochemical, elemental analysis, antidiabetic activity, aqueous pod extract

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INTRODUCTION

Acacia nilotica tree ranges between 5-20 meters with a fissured bark with greyish-pinkish slash, producing low quality redish gum. There are roughly 1,300 species of this plant worldwide with roughly 950 species found in Australia and the rest are found in Africa, Southern Asia and North and South America (Brenan, 1983).

Acacia nilotica contain some psychoactive alkaloids of which dimethyltryptamine (DMT) and N-Methyltryptamine (NMT) are most prominent and useful, other psychoactive compounds present in the plant include tryptamine, β-carbolines, mesculine, bufoteinine and nicotine (Umalkar *et al*., 1976).

Acacia nilotica has been reported to have astringent property and is used by the natives in the treatment of such conditions as impotence, tumor of the eye or testicle, dysentery, leprosy, colds, cough, congestion, fever, haemorrhoids, leucorrhoea, opthalmia, sclerosis, smallpox, tuberculosis and indurations of the liver and spleen. They are also reports of its usage in the treatment of toothache and typhoid (Umalkar *et al*., 1976).

The objective of this study is to evaluate the phytochemical, elemental and the glycaemic activity of the aqueous pod extract of *Acacia nilotica* in wistar albino rats.

MATERIALS AND METHODS

Plant collection and identification: Fresh pods of *Acacia nilotica* were collected in June 2011 from Uba, Askira/Uba Borno State, Nigeria. The pods were identified by a taxonomist in the Department of Biological Sciences, University of Maiduguri, Nigeria. The pods were dried under the shade and ground into fine powder.

Preparation of aqueous extract: Three hundred and fifty grams of the powdered sample were exhaustively extracted with distilled water using reflux method. The crude aqueous extract obtained was concentrated *in vacuo* and a brown colored extract weighing two hundred and sixty three grams (263 g) w/w was obtained. It was thereafter stored in a refrigerator at 4°C until used (Trease and Evans, 1989).

Phytochemical analysis of the extracts of *Acacia nilotica* pod: The aqueous extract and ethyl acetate, N-butanol and residual fractions of *Acacia nilotica* extracts were subjected to qualitative chemical screening for the identification of various classes of active chemical constituents (Clarke, 2008; Odebiyi and Sofowora, 1978; Trease and Evans, 1989).

Test for carbohydrates: A few drops of molisch's reagent was added to 2 mL of the water extract, thereafter small quantity of Conc. Sulphuric acid was added and allowed to form a lower layer. A purple ring at the interface of the liquid indicates the presence of carbohydrates. The mixture was then shaken and allowed to stand for 2 min and diluted with 5 mL of distilled water the presence of a purple precipitate also indicate the presence of carbohydrates (Trease and Evans, 1989; Motohashi *et al*., 2004).

Test for tannins: A portion of the water extracts was diluted with distilled water in a ratio of 1:4 and a few drops of 10% ferric chloride solution was added. A blue or green color indicates the presence of tannins (Trease and Evans, 1989).

Test for saponins: To a small quantity of the powdered sample was added 95% ethanol and boiled. The mixture was filtered and 2.5 mL of the filtrate was added to 10 mL of distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 sec and then it was allowed to stand for over half an hour. Honey comb froth obtained is indicative of the presence of saponins (Sofowara, 1993).

Test for cardiac glycosides: To 10 mL of the extract filtrate was added, a few drops of pyridine and a few drops of 20% sodium hydroxide solutions respectively. A deep red color was indicative of the presence of cardenolide aglycone (Sofowara, 1993).

Test for flavonoids: To 5 mL of 10% sodium hydroxide was added an equal volume of the water extract. A yellow solution indicates the presence of flavonoids (Trease and Evans, 1989).

Elemental analysis: The elemental content was determined using the standard calibration curve method. 0.5 g of air dried sample in an evaporating dish was placed in oven at 80°C and dried to a constant weight. The sample was placed in a weighing crucible and ashed at 500°C in a hot spot furnance for three hours. The ashed material was prepared for determination of trace elements. A portion of Zero point five (0.5 g) grams of the ashed sample was digested by heating for two minutes with a mixture of 10 mL each of Nitric acid (HNO_3), Hydrochloric acid (HCl) and perechloric acid in a 500 mL flask. The aliquot obtained from this study by filteration was mixed in a 10 mL of 2M HNO_3 and 30 mL of distilled water in a 100 mL volumetric flask. The volume was made up to the mark with distilled water. Blank sample and standard

solution for the various elements were similarly done. All samples placed in a plastic container and stored in a refrigerator maintained at 4°C prior to analysis. Flame Emission Spectrometer (FES) (GallenKamp FGA 330) was used to determine sodium and potassium concentration. The other elements, Magnesium, Calcium, Iron, Lead, Zinc, Manganese, Cadmium, Copper and Arsenic were determined by Atomic Absorption Spectrometry (AAS) with SPG Unicam model No. 1 at the appropriate wavelength, temperature and lamp current for each element (Sunderman, 2000; Kolthoff and Elving, 1976).

Induction of diabetes in rats: Thirty five rats were made diabetic by injecting them intraperitoneally with alloxan monohydrate (Sigma-Aldrich Holland) diluted in 0.01 M citrate buffer of pH 4.5 and was administered at the dose rate of 150 mg kg^{-1} b.wt. Fifth (5) days after injection, the fasting blood glucose levels of the rats were determined using one touch glucose meter. The rat's blood glucose level ranged between 110-170 mg dL^{-1} (6.1-8.9 mmol L^{-1}).

Effect of aqueous *Acacia nilotica* pod extract on blood glucose level of normoglycemic albino rats: Twenty five rats of both sexes weighing between 110-200 g were randomly selected and used for this study. They were divided into 5 groups of 5 rats each. Group A (control rats) was administered distilled water, while Groups B-E were administered 200, 400, 600 and 800 mg kg^{-1} of the aqueous extract of *Acacia nilotica*, respectively. Blood glucose level was determined before extract administration and at 0, 1, 6, 12 and 18 h post extract administration using one touch glucose meter (Asatoor and King, 1954).

Effect of aqueous extract of *Acacia nilotica* pod on blood glucose level of diabetic albino rats: Thirty five (35) rats weighing between 110-200 g were used for the study and consist of thirty diabetic rats and five normal rats. The normal rats served as control (Group A), while the thirty diabetic rats were separated into six groups of five rats each (Group B to G). Group A and B were treated with distilled water only while Group C (diabetic rats) was treated with insulin. The diabetic rats in groups D, E, F and G were treated with 200, 400, 600 and 800 mg kg^{-1} body weight of aqueous *Acacia nilotica* pod extract, respectively. Blood glucose levels were determined using one touch glucose meter (Asatoor and King, 1954) at 0, 1, 6, 12 and 18 h post administration of agents.

Blood sample collection: Blood from the tail vein of the rat was collected by snipping part of the tail. Blood was dropped onto the glucose test stripe and blood glucose level was determined using one touch glucose meter.

Statistical analysis: The results are presented as Means±Standard deviations. Differences between means were assessed using Analysis of variance (ANOVA) and post test using Turkey-Kramer multiple comparison test (Mead and Curnow, 1982).

RESULTS AND DISCUSSION

Phytochemical investigation of the aqueous extract and various solvent fractions showed that carbohydrate, tannins, saponins, glycosides and flavonoids were present in the extract. Anthraquinones, alkaloids, terpene and steroids were absent from the extracts. The concentrations of Fe, K and Mn in the *Acacia nilotica* pod extract were within safety limit. However, the concentrations of some other elements such as Zn, Ca, Na, Cd and Cu were much lower than the acceptable levels. Elements like Pb, As and Mo were absent. The Phosphorous and Magnesium concentrations in the pod were 0.49 and 0.39 ppm, respectively. There was significant ($p< 0.05$) decrease by 13.19, 13.95, 11.73, 16.40, 28.80 and 25.46%, respectively in blood glucose level of normal and 14.12, 32.28, 49.67, 38.97 and 74.61% in diabetic rats treated with 400, 600 and 800 mg kg^{-1} of aqueous extract at 12 and 18 h post administration.

Phytochemical analysis of the aqueous pod extract of *Acacia nilotica* revealed the presence of tannins, saponins, flavonoids and carbohydrates. Anthraquinones, Alkaloids, terpene and steroids were not present in the extracts (Table 1). The chemical

Table 1: Phytochemistry of aqueous extract of *Acacia nilotica* pod

Phytochemical constituents	Types of test	Inference
Carbohydrate	Molisch's	+
	Barfoed's	-
	Free reducing sugar	+
	Combined reducing sugar	-
	Ketones	+
	Pentose's	+
Tannins	Ferric chloride	+
	Formaldehyde	+
	Chlorogenic	-
Anthraquinones	Free anthraquinones	-
	Combined anthraquinones	-
Saponins	Frothing	+
Glycosides	General test	+
Terpenes and steroids	Lieberman-Buchard's	-
	Salkowski's	-
Flavonoids	Lead acetate	+
	Sodium hydroxide	-
	Ferric chloride	+
	Pew	+
	Dragendorff's	-
Alkaloids	Mayer's	-

-: Not detected, +: Present in low concentration

constituents present in the extract have been reported to posses many therapeutic values (Robinson, 1987; Frantisek, 1991).

Flavonoids have been reported to possess hypoglycemic and anti-diabetic effect (Ahmad *et al*., 2000). Flavonoids have antioxidant activity, protect cells against oxidative damage and reduce the risk of developing certain types of cancer (Chakarborty *et al*., 1995). Epicatechin flavonoids isolated from *Pterocarpus marsupium* was reported to promote regeneration of β cells of Islets of langerhans in the pancreas (Chakravarthy *et al*., 1980) and on clinical trials, beneficial effect was reported in adult-hood onset diabetes (Ojah *et al*., 1978; Anila *et al*., 2002). Oral administration of flavonoids of the seed of *Cuminum nigrum* caused a significant blood glucose lowering effect both in normoglycemic and alloxan induced diabetic rabbits (Ahmad *et al*., 2000).

Saponins isolated from the leaves of *Acanthopanax senticosus* was reported to have decreased blood glucose of mice in an experimental hyperglycemia induced by injecting adrenaline, glucose and alloxan (Sui *et al*., 1994). Saponins from the fruit of *Balanites aegyptica* were also found to have exhibited prominent anti-diabetic activity in streptozotocin induced diabetic mice (Kalman *et al*., 1991).

Mineral elements serve not only as nutritional sources for both plant and animals but also play other important roles in the environment. Inorganic chemical elements have been shown to be essential in nutrition and are important structural components in cellular processes (WHO, 1993).

Some plants provide mineral elements such as calcium, zinc, magnesium, manganese and copper to the β cells of the liver that enhances insulin production (Aderibigbe *et al*., 1999). The concentration of the trace elements like Pb, Zn, Cd, Cu, As, Ca and Molybdenum (Table 2) were either below the acceptable range, but may still exert some degree of physiological response, though some of these minerals are absent in the pod extract. Iron was the only trace element found within acceptable level (WHO, 1993). Potassium was the only micro element detected within acceptable range; others like Na and P were below the accepted concentrations (Table 2) (WHO, 1993).

Numerous mechanisms of actions have been proposed for different plant species. The hypoglycemic and antidiabetic effect of plants may be due to the presence of insulin-like substances in plants, stimulation of β cells to produce more insulin, high level of fiber which interferes with carbohydrate absorption or the regenerative effect of plants on pancreatic tissue (Chang and Johnson, 1980; Chakravarthy *et al*., 1980; Collier *et al*., 1987; Zarzuelo *et al*., 1990; Shanmugasundaram *et al*., 1990; Abdel *et al*., 1997; Nelson *et al*., 1991).

Hypoglycemic and antidiabetic activity of *Magnifera indica* leaves was associated to reduction in the intestinal absorption of glucose. Similarly *Salvia fruticosa* reduced the glycaemic level in alloxan-induced diabetic rabbits, mainly by reducing intestinal absorption of glucose, without modifying plasma insulin level (Perfumi *et al*., 1991; Aderibigbe *et al*., 1999).

Olea europea L. olive leaf induced hypoglycemia and antidiabetic effect as a result of potentiating the glucose-induced insulin release and increased peripheral uptake of glucose (Gonzalez *et al*., 1992). Some plants extract such as that of *Gymnema sylvestra* produce their anti-diabetic effect by restricting the rise of blood sugar

Table 2: Elemental analysis of the aqueous pod extract of *Acacia nilotica*

Elements	Concentration (ppm)	WHO standard conc. (mg dL^{-1} or ppm)
Iron (Fe)	0.54	0.5-50
Potassium (K)	0.52	0.1-1
Manganese (Mn)	0.50	10-20
Zinc (Zn)	0.48	15-20
Calcium (Ca)	0.40	360-800
Phosphorous (P)	0.49	-
Magnesium (Mg)	0.39	-
Sodium (Na)	0.35	4-5
Cadmium (Cd)	0.09	10-35
Copper (Cu)	0.08	1-3
Lead (Pb)	-	1-2
Arsenic (As)	-	0.02-7
Molybdenum	-	-
-Absent		

Table 3: Effect of aqueous extract of *Acacia nilotica* pod on Mean blood glucose level of normoglycemic albino rats

	Mean blood glucose level (mg dL^{-1})				
	Time (h)				
Treatment Dose (mg kg^{-1})	0	1	6	12	18
Control	77.0±5.68	77.5±5.93 (+0.65)	77.4±5.46 (+0.52)	77.6±7.89 (+0.77)	77.8±5.89 (+1.03)
200	85.6±1.78	86.2±1.99 (+0.70)	92.6±4.43 (+7.55)	78.2±8.21 (-9.46)	77.8±3.98 (-10.03)
400	76.8±4.48	82.4±4.48 (+6.79)	87.6±3.79 (+12.32)	75.8±3.90[b] (-13.19)	67.4±4.30[b] (-13.95)
600	72.4±4.16	70.8±2.71 (-2.26)	68.6±5.61 (-5.54)	64.8±4.99 (-11.73)[b]	62.2±5.41[b] (-16.40)
800	81.2±3.25	77.4±4.32 (-4.91)	79.8±4.43 (-1.75)	63.0±4.02[b] (-28.80)	64.8±1.93[b] (-25.46)

Data is as Mean±SD, b: Significantly ($p<0.05$) lower than zero hour value Numbers in bracket indicates percentage increase (+) or decrease (-) in blood glucose level when compared with zero hour

Table 4: Effect of aqueous pod extract of *Acacia nilotica* on Mean blood glucose level of diabetic albino rats

	Mean blood glucose level (mg dL^{-1})				
	Time (h)				
Treatment Dose (mg kg^{-1})	0	1	6	12	18
Control	77.0±6.63	77.2±5.93 (+ 0.26)	77.6±6.40 (+ 0.77)	77.8±6.54 (+ 1.03)	78.2±2.34 (+1.53)
Insulin	358.0±79.06	195.4±99.15 (-83.20)	199.6±96.25 (-79.35)	233.6±100.9 (-53.25)	240.0±99.11 (-49.17)
200	338.0±23.11	345.4±25.74 (+2.14)	344.0±23.37 (+1.74)	353.4±21.29 (+4.36)	351.4±17.73 (+3.81)
400	360.4±101.7	394.2±79.46 (+8.57)	439.0±87.74 (+1.79)	389.4±89.17 (+7.45)	315.8±94.83[b] (-14.12)
600	462.2±38.52	480.6±53.49 (+3.82)	490.4±61.50 (+5.75)	349.4±94.82[b] (-32.28)	308.8±98.88[b] (-49.67)
800	271.0±40.44	351.6±51.66 (+22.92)	362.6±56.10 (+25.26)	195.0±18.29[b] (-38.97)	155.2±5.51[b] (-74.61)
Diabetic untreated	333.2±34.02	366.8±63.7 (+9.16)	435.6±78.47 (+23.51)	455.4±52.86 (+26.83)	475.2±74.41 (+29.88)

Data is as Mean±SD, b: Significantly ($p<0.05$) lower than zero hour value Numbers in bracket indicates percentage increase (+) or decrease (-) in glucose level when compared with zero hour

caused by pituitary hormones responsible for inhibiting peripheral utilization of glucose as well as glycogenolysis in maturity onset diabetes (Yususf *et al*., 1994).

The treatment of albino rats with graded doses (400, 600 and 800 mg kg^{-1}) of aqueous *Acacia nilotica* pod extract produced significant ($p<0.05$) dose dependent hypoglycemic and antidiabetic effect especially at higher doses (600 and 800 mg kg^{-1}), 12-18 hours post administration (Table 3, 4). The hypoglycemic and antidiabetic effect of *Acacia nilotica* pod extract may be due to the presence of saponins and flavonoids in the aqueous pod extract. The antidiabetic effect of the aqueous pod extract of *Acacia nilotica* may not be by stimulating the production or release of insulin since alloxan used in this experiment is known to destroy the β cells of Islets of langerhans of the pancreas, but may be by reducing intestinal absorption of glucose or increasing the peripheral utilization of glucose as has been exhibited by *Magnifera indica* Pour (1997).

CONCLUSION

Conclusively it has been deduced from the *In vivo* study that the aqueous pod extract of *Acacia nilotica* can be used in the management of chronic hyperglycemic conditions considering the period of onset of action of the product (12-18 h) when compared to the standard drug (Insulin) that showed glucose reducing effect immediately post administration.

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