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Antidiabetic Potentials of Aqueous Extracts of *Acacia nilotica* (Fabaceae), *Anisopus manni* (Asclepiadaceae) and a Recipe Comprising the Two Plants in Experimental Rats

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ABSTRACT

The seeds of *Acacia nilotica* (Leguninosae), and leaves of *Anisopus manni* (Asdepiadaceae) are prescribed traditionally as remedy for diabetes mellitus both individually and in combination as a recipe. This study evaluated the hypoglycaemic and antidiabetic activity of the aqueous extracts of the individual parts of the plants and their recipe in experimental rats. Aqueous extracts of the different plants parts and their recipe were orally administered to normoglycaemic, glucose-induced hyperglycaemic and alloxan-induced diabetic rats and their blood glucose monitored over a period of 4 hours. The *Acacia nilotica* aqueous extract at a dose of 300 mg/kg significantly ($p<0.05$) lowered the fasting blood glucose levels of the different experimental groups by 55.63%, 62.55%, 39.27%, respectively. For *Anisopus manni* aqueous extract the percentage reductions recorded were 59.4%, 61.0% and 56.5% respectively. The recipe of both plants produced a maximum fasting blood glucose reduction of 60.5%, 54.2%, and 36.5% for the groups respectively. The reductions observed when *Acacia nilotica*, *Anisopus manni* and the recipe were administered to alloxan-induced diabetics rats were 39.27%, 56.6%, and 36.5% respectively and were all higher than the 32.27% reduction seen in diabetic rats administered with the standard drug, glibenclamide. The study confirms the antidiabetic property of the individual plants and the recipe through a mechanism that can be both pancreatic and extra pancreatic.

Keywords: Antidiabetic, *Acacia nilotica*, *Anisopus manni*, recipe, blood glucose, Diabetes Mellitus.

Introduction

Diabetes mellitus (DM) has today remained one of the world's greatest medical challenges. It is a metabolic disorder caused by inadequate insulin secretion and/or action leading to increased blood glucose levels (hyperglycaemia). World diabetes prevalence is estimated at over 400 million people representing some 8% of the population, while for Africa the prevalence rate is as high as 4.5%.[1] Mortality, morbidity and economic burden for the disease worldwide is high. Mortality is estimated at 5.0 million deaths per year globally and the economic burden stands at over 600 million dollars spent on the disease yearly.[2]

Complications of the disease that leads to the mortality and the economic burden includes nephropathy, neuropathy, angiopathy, amputations and blindness.[3] Factors associated with the increase in diabetes prevalence include sedentary life styles, obesity, probable genetic factors and unhealthy eating habits.[4] Dietary regulation with exercise, oral hypoglycaemic drugs and insulin injections[5] remain the major management strategies for the disease by orthodox medical practitioners. Side effects, compliance and costs, thereby leading to complications, necessitate the search for safer and more efficacious antidiabetic agents, especially from medicinal plants.

Acacia nilotica (Fabaceae/Leguminosae) is a multipurpose widespread tree in Africa. It is an established economic tree because of its use as a source of tannins, gums, timber, fuel and animals' fodder.[6] Its economic uses also include its use in paper production with similar pulping properties with other tropical timbers.[7] Medicinal uses ascribed to the plant are varied and wide. Traditionally, the bark, leaves, pods and flowers are used to treat fever, dysentery, bleeding piles and menstrual problems.[8] The root is prescribed for tuberculosis, the wood for small pox and the leaves for ulcers.[9] The pods are considered very useful in folk medicine to treat diabetes mellitus.[10]

Anisopus manni (Asclepiadaceae) is a glabrous twining shrub, a strong climber and widely distributed in Africa.[11] Aqueous extract of the stem bark of this shrub is reported to have potential hypoglycaemic effect.[12]

Evaluations of the two plants singly for possible antidiabetic activity have been reported,[10,12] but achieving complete glycaemic control is still a challenging task. However, the two plants as a recipe is also claimed to manage diabetes mellitus here in Northern Nigeria. Polytherapy with two or more hypoglycaemic plants may lead to achieving the much needed glycaemic control, more importantly polyherbal therapy is the normal medicinal practice to treat most diseases by traditional medical practitioners.

In this study we report the possible antidiabetic effects of aqueous extracts of the seeds of *A. nilotica* and whole plant of *A. manni* singly and as a recipe in normoglyaemic, glucose-induced hyperglycaemic and alloxan-induced diabetic rats.

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Materials and Methods

Collection of Plants Materials

Acacia nilotica (seeds) and *Anisopus manni* (whole plant) were collected in Maiduguri, Borno State, Nigeria in August 2016. The plants were identified and authenticated at the Department of Biological Science, University of Maiduguri. Voucher specimens CHM/11-009 and CHM/14-005 for *A. nilotica* and *A. manni*, respectively were deposited in the herbarium of University of Maiduguri Research Laboratory for future reference. They were shade-dried and processed into powder before use.

Aqueous Extracts Preparations

Aqueous extracts of *A. nilotica*, and *A. manni* were prepared by boiling 150 g powder of each in 1 L of water for 10 min. For the recipe, 75 g of each powder were mixed together and boiled in 1 L of water for 10 min. The extracts were filtered, evaporated and concentrated under reduced pressure.

Experimental Animals

Wister rats of both sexes weighing 150-250g were used for the study. The rats were obtained from the Animal House of the Department of Biochemistry, Faculty of Science, University of Maiduguri, Borno State, Nigeria. They were maintained under standard laboratory conditions, fed with standard rodent diet and tap water *ad libitum*. The rats were treated in accordance with the principles of laboratory animal care.[13]

Experimental Design

The study was carried out on normoglycaemic, glucose-induced hyperglycaemic and alloxan-induced diabetic rats. The rats were fasted for 18 h before each experiment and blood was collected from tip of the rats tails for glucose assay.

Study on normoglycaemic rats

Fasting blood glucose concentration was first determined in 18-hour fasted rats. The aqueous extracts of *A. nilotica* (300mg/kg body weight), *A. manni* (200mg/kg bw) and the recipe (300 mg/kg bw) were administered orally (using BMI feeding tube, size 8) to the rats and their blood glucose concentration monitored at 0.5, 1, 2, 3 and 4 h. Normal control rats were administered with distilled water in place of the extracts. Positive control group rats received glibenclamide (2 mg/kg).[14]

Study on glucose-induced hyperglycaemic rats (Oral Glucose Tolerance Test, OGTT)

The oral glucose tolerance test was done in normal rats after 18-hour fast. The test groups were orally administered with doses of the extracts simultaneously with glucose (2 g/kg) or the glucose administered 30 minutes after administration of the extracts. Negative control group received distilled water and positive control groups were administered with glibenclamide (2 mg/kg). Blood glucose concentration was monitored over a period of 4 hours.

Study on Alloxan-induced Diabetic rats

Diabetes was induced in overnight-fasted rats by a single intramuscular (at base of tail) injection of 120 mg/kg alloxan monohydrate (Sigma, Aldrich) dissolved in cold normal saline. After five days, rats with fasting blood glucose concentration of more than 200 mg/dL were considered as alloxan-induced diabetic rats.

The extracts were then administered to the diabetic rats. Normal and negative controls were administered distilled water and positive controls were administered with glimbenclamide. Blood glucose concentrations were also monitored at the same interval and for the same duration.

Blood Glucose Determination

Drops of blood from tip of rats tails was collected in fluoridated eppendoff tubes and plasma harvested by centrifugation at 3000g for 10 minutes. Glucose concentration was assayed using glucose oxidase kit (Randox, UK) which is based on the principle of Trinder, 1959.[15]

Statistical analysis

The results are presented as Mean ± SEM of five replicate determinations. Students T-test was used to statistically analyze the differences between two means and differences were considered significant at $P < 0.05$.

Results and Discussion

The effects of the different aqueous extracts on normoglycaemic, glucose-induced hyperglycaemic and alloxan-induced diabetic rats are presented in tables 1, 2 and 3, respectively. In the normoglycaemic rats study, all the extracts significantly reduced the blood glucose concentrations of the different groups. Percentage reductions of 55.6%, 59.4% and 60.5% were seen after 4 hours of administering the different doses of *A. nilotica, A. manni* and their recipe, respectively. The percent reduction of 37.6% seen in the rats administered the standard drug glibenclamide was much lower than the reductions seen in the test groups.

The maximum percentage reduction in fasting blood glucose of the alloxan-induced diabetic rats was seen in the group orally fed with the aqueous extract of *A. manni* (Table 3). The recipe and *A. nilotica* extracts also significantly lowered the fasting blood glucose concentrations of rats in their groups and the reductions were comparable with the glibenclamide treated group.

The study compared the antidiabetic activity of a recipe traditionally prescribed for diabetes mellitus and the two plants constituting the recipe. The recipe is a combination of dry powder of seeds of *A. nilotica* and *A. manni* whole plant. Different parts of the two plants have separately been reported to possess antidiabetic activity. The aqueous extracts of the leaf[16-17] and stem bark[18]of *A. nilotica* have been reported to possess antidiabetic activity. *Anisopus manni* methanol leaf extract[19] and aqueous stem extract[12] have also been reported for antidiabetic potentials, the present study also demonstrated potentials of the individual plants to lower fasting blood glucose. Both plants separately lowered fasting blood glucose concentration of alloxan-induced diabetic rats significantly ($P<0.05$). The *A. nilotica* showed a maximum percent reduction of 51.9% which was statistically similar with the 50.6% reduction seen in the alloxan-induced diabetic rats administered the *A. manni* aqueous extract. The recipe, on the other hand, produced a percentage reduction of 36.5% and was significantly lower than the reductions seen in the groups administered the individual plants, but was comparable with the 32.6% reduction seen in the diabetic rats treated with the standard drug glibenclamide.

The result clearly demonstrated the antidiabetic potential of the recipe though about 0.5-fold lower than for each of the individual plants. The study is an acute one involving a single oral administration of the extracts. It requires at least a sub-chronic study, where the features of diabetes will clearly manifest, before a definite conclusion can be drawn on the superiority of either the recipe or the individual plants.

Treatments of normal rats with the different extracts produced statistically similar hypoglycaemic effects and were significantly higher than the effect seen in normal rats treated with the standard drug. That the extracts possess both hypoglycaemic and antihyperglycaemic effects is a common feature of many reported antidiabetic medicinal plants.[20-22]

The hyperglycaemia in normal rats challenged with 2 g/kg glucose load was restored back to normal within 2 hours (Table 2). *A. nilotica* and the recipe aqueous extracts inhibited the rise in blood glucose levels when administered simultaneously with the glucose or 30 minutes before the glucose. However, the *A. manni* whole plant aqueous extract inhibited the rise in blood glucose only when administered 30 minutes before glucose

Oral glucose tolerance test measures the body's response to glucose challenge and it is used in experimental animals to screen medicinal plants for possible antihyperglycaemic activity.[23-24] Impaired oral glucose tolerance is indicative of predisposition to diabetes mellitus; therefore agents capable of bringing down blood glucose concentration to within normal limits after a glucose challenge are regarded as good antidiabetic candidates since they are likely to arrest the progression of the impaired glucose tolerance to diabetes.[25] In this study, the glucose was administered either simultaneously with the extract or 30 minutes after the extract administration.

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Table 1: Effect of Oral Administration of Aqueous Extracts of *Acacia nilotica* seeds, *Anisopus manni* (whole plant), Recipe comprising *Acacia nilotica* (seeds), *Anisopus manni* (whole plant) on fasting blood glucose concentration (mg/dL) in *Normoglycaemic* rats.

Treatment Group	Time (hours)					
	0	**0.5**	**1**	**2**	**3**	**4**
Normoglycaemic Rats + Water	56.44 ± 5.27	94.04 ± 14.83	81 ± 4.22	112.43 ± 8.35	79.66 ± 3.69	77.84 ± 5.42
Normoglycaemic Rats + *Acacia nilotica*	81.85 ± 13.40	73.35 ± 9.83 (10.38%)	72.57 ± 0.00 (11.34%)	67.30 ± 7.06 (17.78%)	51.54 ± 5.81* (37.03%)	36.32 ± 3.81* (55.63%)
Normoglycaemic Rats + *Anisopus manni*	68.96 ± 7.31	86.22 ± 5.890	45.30 ± 6.753 (34.31%0	36.66 ± 5.51* (46.84%)	36.00 ± 2.51* (46.84%)	28.02 ± 2.86* (59.37%)
Normoglycaemic Rats + *Recipe*	61.44 ± 9.75	61.46 ± 4.85	61.46 ± 4.13	53.34 ± 5.50* (13.18%)	43.66 ± 7.42* (28.94%)	24.25 ± 5.10* (60.53%)
Normoglycaemic Rats + *Glibenclamide*	77.72 ± 6.78	58.20 ± 6.84 (25.12%)	50.44 ± 7.11 (35.10%)	46.63 ± 3.73 (40.00%)	46.63 ± 3.73 (40.00%)	48.50 ± 6.59 (37.60%)

Values are expressed as Mean ± SEM, n = 5. Values in parenthesis are percentage decrease in blood glucose concentrations.
* Significantly lower ($p < 0.05$) compared with 0 hour in the same group.

Table 2: Effect of Oral Administration of Aqueous Extract of *Acacia nilotica* seeds, *Anisopus manni* (whole plant) and a Recipe comprising *Acacia nilotica* (seeds) and *Anisopus manni* (whole plant) on fating blood glucose concentration (mg/dL) in glucose- induced hyperglycemic rats.

Treatment Group	Time (hours)					
	0	**0.5**	**1**	**2**	**3**	**4**
Glucose Induced Rats + Water	72.72 ± 9.29	100.88 ± 14.23	128.04 ± 11.62	85.36 ± 14.46	69.84 ± 13.13	54.32 ± 12.84
Glucose Induced Rats + *Acacia nilotica* simultaneous	60.67 ± 2.63	58.20 ± 4.69 (4.07%)	48.50 ± 3.09 (20.06%)	41.57 ± 2.19 (31.48%)	24.94 ± 4.69* (58.90%)	15.23 ± 2.59* (74.90%)
***Acacia nilotica* + Glucose (30 min after)**	78.80 ± 2.42	70.33 ± 5.93 (10.75%)	67.90 ± 11.22 (13.83%)	53.34 ± 12.57 (32.30%)	57.30 ± 4.69 (27.28%)	55.78 ± 9.83 (29.21%)
Glucose Induced + *Anisopus manni* simultaneous	70.33 ± 11.12	116.40 ± 19.78 (65.50%)	111.50 ± 16.46 (58.54%)	87.61 ± 6.17 (24.57%)	41.22 ± 6.10 (41.39%)	32.85 ± 3.72 (53.29%)
***Anisopus manni* + Glucose (30 min after)**	82.44 ± 14.04	94.55 ± 5.92 14.69%	75.18 ± 10.44 (8.81%)	48.50 ± 10.15 (41.17%)	33.95 ± 5.93* (58.82%)	31.53 ± 4.84* (61.75%)
Glucose Induced + Recipe simultaneous	72.75±13.10	81.58 ± 13.44 (12.14%)	45.45 ± 9.06* (37.53%)	30.35 ± 6.05* (58.28%)	36.40±4.94* (49.97%)	33.35 ± 5.79* (54.16%)
Recipe + Glucose (30 min after)	80.16±2.13	88.38 ± 6.27 (10.25%)	92.70 ± 8.74 (15.64%)	30.16 ± 5.26 (62.38%)	60.36±11.06 (24.70%)	64.66 ± 9.62 (19.34%)
Glucose Induced + Glibenclamide	80.03±6.17	87.61 ± 6.17 (09.50%)	113.98 ± 6.17 (42.42%)	111.55 ± 6.17 (39.40%)	92.15 ± 6.17 (15.14%)	65.47 ± 6.17 (18.19%)

Values are expressed as Mean ± SEM, n = 5. Values in parenthesis are percentage increase in blood glucose concentrations.
* Significantly lower ($p < 0.05$) compared with 0 hour in the same group.

Table 3: Effect of Oral Administration of Aqueous Extract of *Acacia nilotica* seeds, *Anisopus manni* (whole plant) and a Recipe comprising *Acacia nilotica* (seeds) and *Anisopus manni* (whole plant) on fasting blood glucose concentration (mg/dL) in Alloxan-Induced diabetic rats.

Treatment Group	**Time (hours)**					
	0	**30 min**	**1**	**2**	**3**	**4**
Alloxan-Induced diabetic +	299.07 ± 4.11	291.80 ± 6.42	287.77 ± 3.29	291.93 ± 6.55	284.53 ± 3.29	274.83 ± 2.12
Water		(2.43%)	(3.78%)	(2.39%)	(4.86%)	(8.11%)
Alloxan-Induced diabetic +	228.18 ± 11.80	225.18 ± 11.80	197.47 ± 6.64*	176.68 ± 6.63*	152.53 ± 5.66*	138.57 ± 5.60*
Acacia nilotica		(1.31%)	(13.46%)	(22.57%)	(33.15%)	(39.27%)
Alloxan-Induced diabetic +	215.80 ± 4.65	208.50 ± 4.65	181.80 ± 6.25	156.45 ± 5.04*	130.90 ± 6.26*	106.70 ± 8.86*
Anisopus manni		(3.38%)	(15.76%)	(27.50%)	(39.34%)	(56.56%)
Alloxan-Induced + Recipe	224.23 ± 7.83	221.28 ± 7.36	212.19 ± 7.83*	190.97 ± 5.81*	163.69 ± 7.83*	142.47 ± 5.81*
		(1.32%0	(5.37%)	(14.83%)	(27.00%)	(36.46%)
Alloxan-Induced +	254.62 ± 7.14	221.37 ± 9.15*	216.40 ± 11.62*	208.95 ± 6.20*	191.50 ± 2.55*	171.63 ± 4.38*
Glibenclamide		(13.06%)	(15.01%)	(17.94%)	(24.79%)	(32.59%)

Values are expressed as Mean ± SEM, n = 5. Values in parenthesis are percentage decrease in blood glucose concentrations.
* Significantly lower ($p < 0.05$) compared with 0 hour in the same group.

Aqueous extracts of both *A. nilotica* and the recipe significantly inhibited rise in blood glucose concentration whether administered simultaneously or 30 minutes before the glucose, suggesting that their antihyperglycaemic activity maybe by both pancreatic and extra pancreatic.[26] The *A. manni* on the other hand inhibited rise in blood glucose concentration only after its administration 30 minutes before the glucose load. *A manni* antihyperglycaemic activity therefore maybe through direct stimulation of insulin secretion from pancreatic β cells, a common mechanism of many antidiabetic medicinal plants.[27-29]

Conclusion

In conclusion the acute study confirms the antidiabetic property of the recipe through a mechanism that can be both pancreatic and extra pancreatic. A sub-chronic study is however required to establish the most effective antidiabetic between the recipe and the individual plants.

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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