

The Hypoglycemic Activity and Safety of Aqueous Stem Bark Extracts of *Acacia nilotica*

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Abstract

Acacia nilotica is used traditionally to manage several diseases including Diabetes mellitus, however, its efficacy and safety is not well evaluated. The aim of this study was to determine *in vivo* the hypoglycemic activity and safety of the aqueous stem bark extracts of this plant in male swiss white albino mice. The anti-diabetic activity was screened in alloxan induced diabetic mice using oral and intra-peritoneal routes. The safety of this plant extract was studied in mice that were orally and intraperitoneally administered with 1 g/kg body weight daily for 28 days by recording changes in body and organ weight, hematological and biochemical parameters and histology. Mineral composition was estimated using total reflection X-ray fluorescence system and atomic absorption spectrometry. Phytochemical composition was assessed using standard procedures. The extract administered at 50, 100, 200, 300 mg/kg body weight showed hypoglycemic activity. The intraperitoneal route was more effective compared to the oral route. Intraperitoneal administration of the extract at 1 g/kg body weight significantly reduced body weight gain, percent organ to body weight of testes, while oral administration at the same dose decreased levels of platelets. Oral administration of the aqueous stem bark extracts of *A. nilotica* at 1 g/kg body weight caused increase in levels of γ -glutamyl transpeptidase, Creatine kinase, and Total bilirubin while decreasing levels of alanine transaminase, aspartate aminotransferase, α -Amylase, and Alkaline phosphatase. Intraperitoneal administration of the same dose decreased levels of aspartate aminotransferase. The aqueous stem bark extract of *A. nilotica* contained tannins, total phenols, flavonoids, saponins, and alkaloids. Sodium, chloride, potassium, calcium, titanium, vanadium, chromium, manganese, iron, copper, zinc, arsenic, nickel, lead, and cadmium were present in the aqueous stem bark extracts of *A. nilotica* at levels below the recommended daily allowance. In conclusion, the observed hypoglycemic activity and slight toxicity could be associated with the phytochemicals present in this plant extract.

Keywords: Diabetes mellitus; *Acacia nilotica*; Hypoglycemic activity; Antidiabetic; Mineral composition; Phytochemicals; Toxicity

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both [1]. The basic effect of insulin lack or insulin resistance on glucose metabolism is inefficient glucose uptake and utilization by most cells of the body except those of the brain. As a result blood glucose concentration increases, cell utilization of glucose falls and utilization of fat and protein increases [2].

International Diabetes Federation (IDF) estimates that more than 366 million people have diabetes worldwide and this is expected to rise to 552 million by 2030. The number of people living with diabetes in Africa has now risen to 14.7 million. This is expected to increase to 28 million by 2030, around a 90% increase [3]. In Kenya a study in 2009 showed a prevalence of 4.2% in the general population, with a prevalence of 2.2% of the rural areas and a prevalence of 12.2% in the urban population [4].

The vast majority of cases of diabetes fall into two broad etiopathogenic categories. In one category, type I diabetes, the cause is an absolute deficiency of insulin secretion. In the other much more prevalent category, type II diabetes, the cause is a combination of resistance to insulin action and an inadequate compensatory insulin secretory response [1].

The major option in diabetes mellitus therapy is lifestyle management. Besides exercise, weight control and medical nutrition therapy [5]. Patients with type I diabetes always require treatment with exogenous insulin. For Type II diabetes, treatment options

begin with diet modification and lifestyle interventions but often oral hypoglycemic agents or insulin or both are required as the disease progresses [6].

Although insulin has been designated an essential drug by WHO, it is not yet universally accessible to all those who need it in the majority of countries of the world. Continuous access to insulin remains a major problem in many developing countries especially those in sub-Saharan Africa [7]. Presently, there is a growing interest in herbal remedies due to the side effects associated with the oral hypoglycemic agents (therapeutic agent) and insulin for the treatment of diabetes mellitus [8].

The World Health Organization (WHO) estimates that up to 80% of the world's population, mostly in developing countries, relies on traditional medicine practices for its health care needs [9]. A notable number of modern drugs have been synthesized from these natural medicinal plants. These drugs are based on the indigenous medicinal information of plants. This natural source has been used to cure various diseases throughout the world. Actually, plants have great diversity of

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bioactive compounds and this is an indication which makes plants a prosperous source of different types of drugs [10].

Several species of herbal drugs have been described in the scientific and popular literature as having anti-diabetic activity [8]. In diabetes, some herbal alternatives are proven to provide symptomatic relief and assist in the prevention of the secondary complication of the disease. Some herbs have also been proven to help in regeneration of β -cells and in overcoming resistance. In addition to maintaining normal blood sugar level, some herbs are also reported to possess antioxidant activity and cholesterol lowering action [11].

Traditional anti-diabetic plants might provide a useful source of new oral hypoglycemic compounds for development as pharmaceutical entities, or as simple dietary adjuncts to existing therapies [12].

Acacia nilotica is a common, medium sized tree, belongs to the family Mimosaceae and used traditionally to treat Gonorrhoea, chest pain and cough [9]. The pods of *A. nilotica* are fed to cattle to increase the milk yield and also used ethno-medicinally for the treatment of skin diseases, stomach ache, malaria, sore throat as well as for tooth problems [13]. Stem bark extract of *Acacia nilotica* exhibits antioxidant properties [14].

Water and methanol extracts of different parts of *Acacia nilotica* (leaves and stem bark) exhibited significant anti-bacterial activity against both gram positive and gram negative pathogens, and also showed good antifungal activity against some fungal strains [13]. The anti-diabetic effect of *Acacia nilotica* has been investigated in Pakistan and it has shown good hypoglycemic and hypolipidemic activity [15].

Materials and Methods

Plant samples

The plant used in this study was collected from its natural habitats in Machakos County, Kenya on the basis of ethno botanical information. An acknowledged authority in taxonomy authenticated the botanical identity of the plant and a voucher specimen was deposited at the National Museums of Kenya Herbarium, Nairobi. A traditional medical practitioner provided the information on which plant to collect, what part to collect, and the precise locality where it grows.

Initial processing of the plants

The part of the plant collected was stem bark. The stem was harvested and the bark peeled off while still fresh, cut into small pieces and then dried at room temperature for 1 month. The stem bark was ground when completely dry using an electric mill. The powdered plant material was labelled kept at room temperature away from direct sunlight in closed dry plastic bags.

Preparation of the aqueous extract

One hundred grams of the powdered plant material was extracted in 1 liter distilled-deionized water at 60°C for 6 hour. The mixture was left to cool at room temperature and then decanted into dry clean conical flask through folded cotton gauze stuffed into a funnel. The decanted extract was then filtered using Whatman number one filter papers under vacuum pump. The filtrate was then freeze-dried for 72 hour. The freeze-dried powder was then weighed and stored in airtight container at -20°C until used for bioassay.

Experimental animals

The study used male Swiss White Albino mice (3-4 weeks old) that

weighed 23-27 g with a mean weight of 25 g. These were bred in the Animal house at the Department of Biochemistry and Biotechnology of Kenyatta University. The mice were housed at a temperature of 25°C with 12 hours/12 hours darkness photoperiod and fed on rodent pellets and water *ad libitum*. The experimental protocols and procedures used in this study were approved by the Ethics Committee for the Care and Use of Laboratory Animals of Kenyatta University, Kenya.

Induction of hyperglycemia

Hyperglycemia was induced experimentally by a single intraperitoneal (IP) administration of 186.9 mg/kg body weight of a freshly prepared 10% alloxan monohydrate (2,4,5,6 tetraoxypyrimidine; 5-6-dioxyuracil) obtained from Sigma (Steinheim, Switzerland) [16].

Forty-eight hours after alloxan administration, blood glucose level was measured using a glucometer. Mice with blood glucose levels above 2000 mg/L were considered diabetic and used in this study. Prior to initiation of this experiment, the animals were fasted for 8-12 hours [17] but allowed free access to water until the end of this experiment.

Experimental Design

For either intraperitoneal or oral route of drug administration, the experimental mice were randomly divided into seven groups of five animals each. Group I consisted of normal mice either intraperitoneally or orally administered with 0.1 ml physiological saline; Group II consisted of alloxan induced diabetic mice either intraperitoneally or orally administered with 0.1ml physiological saline; Group IIIa consisted of alloxan induced diabetic mice intraperitoneally administered with 0.025 insulin units (0.25 insulin units in 1 ml) (1 IU/kg body weight) in 0.1 ml physiological saline; Group IIIb consisted of alloxan induced diabetic mice orally administered with 0.075 mg glibenclamide (0.75 mg in 1 ml) (3 mg/kg body weight) in 0.1 ml physiological saline; Group IV consisted of alloxan induced diabetic mice either intraperitoneally or orally administered with 1.25 mg extract (12.5 mg in 1 ml physiological saline) (50 mg/kg body weight) in 0.1 ml physiological saline; Group V consisted of alloxan induced diabetic mice either intraperitoneally or orally administered with 2.5 mg extract (25 mg extract in 1 ml physiological saline) (100 mg/kg body weight) in 0.1 ml physiological saline; Group VI consisted of alloxan induced diabetic mice either intraperitoneally or orally administered with 5 mg extract (50 mg extract in 1 ml physiological saline) (200 mg/kg body weight) in 0.1 ml physiological saline; Group VII consisted of alloxan induced diabetic mice either intraperitoneally or orally administered with 7.5 mg extract (75 mg extract in 1 ml physiological saline) (300 mg/kg body weight) in 1 ml physiological saline. 0.1 ml of either insulin or glibenclamide or the plant extract solution was administered either intraperitoneally or orally to each experimental mouse.

Blood sampling and glucose determination

Blood sampling was done by sterilizing the tail with 10% alcohol and then nipping the tail at the start of the experiment and repeated after 1, 2, 3, 4, 6 and 24 hours. Bleeding was enhanced by gently "milking" the tail from the body towards the tip. After the operation, the tips of the tail were sterilized by swabbing with 70% ethanol. The blood glucose levels were determined with a glucose analyser model (Hypoguard, Woodbridge, England).

In vivo single dose toxicity test

The mice were randomly divided into four different groups of

five mice each. Group I and II consisted of untreated control mice intraperitoneally and orally, respectively, administered daily for 28 days with 0.1 ml physiological saline. Group III and IV consisted of normal mice intraperitoneally and orally respectively daily for 28 days with (1 g/kg body weight) of the aqueous stem bark extracts of *Acacia nilotica* in 0.1 ml physiological saline.

During this period, mice were allowed free access to mice pellet and water and observed for any signs of general illness, change in behaviour and mortality. At the end of 28 days, the mice were sacrificed.

Determination of body and organ weight

The body weight of each mouse was assessed after every seven days during the dosing period up to and including the 28th day and the day of sacrifice (day zero, 7, 14, 21, 28). On the day of sacrifice, all the animals were euthanized using chloroform as an inhalant anaesthesia and blood samples were drawn from the heart of each sacrificed mouse. The blood samples were collected in plastic test tubes and divided into two portions. One portion was used for determination of hematological parameters. The other portion was allowed to stand for 3 hours to ensure complete clotting. The clotted blood samples were centrifuged at 3000 rpm for 10 min and clear serum samples were aspirated off and stored frozen at -20°C for metabolite and enzyme assays. The liver, kidney, heart, lungs, spleen, intestine, brain and testis were carefully dissected out, weighed and preserved in 10% neutral buffered formalin for histological analysis.

Determination of hematological parameters

Blood parameters and indices were determined using standard protocols [18]. Red blood cells count (RBC), white blood cells count (WBC), hemoglobin (Hb), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), packed cell volume (PCV), mean corpuscular volume (MCV) and platelets (PLT) were determined in whole blood with EDTA anticoagulant using the Coulter Counter System (Beckman Coulter®, ThermoFisher, UK).

Differential white blood cell count for neutrophils, lymphocytes, eosinophils, basophils and monocytes were determined from giemsa stained blood films using a hemocytometer [18]. Air-dried thin blood films stained with giemsa stain were examined microscopically using magnification 400 and 1000 for differential WBC counts and cell morphology, respectively.

Laboratory determination of biochemical parameters

The biochemical parameters determined on the sera specimen using the Olympus 640 Chemistry AutoAnalyser were aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), lactate dehydrogenase (LDH), creatine kinase (CK), α -amylase (α -AMYL), total bilirubin (T-BIL), direct bilirubin (D-BIL), urea and creatinine. All reagents for the machine were commercially procured to fit the required volumes and concentrations. The reagents were in specific containers referred to as reagent cartridges. The reagent cartridges were bar coded for the identification by the machine. The machine was programmed for the selected tests for each sample. The sample sectors were then placed into the autoloader assembly. A number of events that occurred simultaneously were performed automatically under the direct control of the instrument microprocessor. All the assays were performed based on the standard operating procedures (SOPs) written and maintained in the Department of Laboratory Medicine, Kenyatta National Hospital.

Histopathology

The formalin fixed tissues were processed using standard histopathology protocol and stained with haematoxylin and eosin for microscopy. The stained tissues were cover slipped with DPX, dried and examined microscopically for any pathological changes [19].

Qualitative analysis on phytochemical constituents

Qualitative analysis of the phytochemicals was done following the recommended procedures: Alkaloids [20], Saponins [21], flavanoids [22], phenolics [21] and tannins [22].

Quantitative analysis on phytochemical constituents

The Phytochemicals were also quantified using standard procedures: Alkaloids [23], Saponin [24], flavonoids [25], phenols [26] and tannins [27].

Mineral elements analysis

Mineral composition of the plant extracts was analyzed using total reflection X-ray fluorescence system (TRXF) and atomic absorption spectrometry (AAS). TRXF system was used to determine the content of Sodium (Na), Magnesium (Mg), chlorine (Cl), potassium (K), Calcium (Ca), Titanium (Ti), Vanadium (V), manganese (Mn), iron (Fe), Copper (Cu), Zinc (Zn), Gallium (Ga), Arsenic (As), selenium (Se), Bromine (Br), Rubidium (Rb), Strontium (Sr), Nickel (Ni), Lead (Pb), and Uranium (U) in the lyophilized plant samples as described by [28]. Atomic absorption spectrometry (AAS) was used for the analysis of Magnesium, Chromium and Cadmium [29]. All the analysis was processed following the instructions from the manufacturer.

Data management and statistical analysis

The Data was entered in the Microsoft Excel Spread Sheet, cleaned and then exported to Statistical Package of Social Sciences (SPSS) Software for analysis. Results were expressed as Mean \pm Standard Deviation (SD) of the number of animals used per every study point. Statistical analysis were done using ANOVA and post-ANOVA to compare the means of untreated normal control mice with diabetic mice treated with saline, diabetic mice treated with the conventional drug, and diabetic mice treated with plant extracts at doses of 50 mg/kg body weight, 100 mg/kg body weight, 200 mg/kg body weight, and 300 mg/kg body weight. For *in vivo* toxicity test student unpaired t-test was used to compare the data of normal control group with the group treated with the extract. $p \leq 0.05$ was considered statistically significant.

Results

Effect of oral and intraperitoneal administration of stem bark extracts of *Acacia nilotica* on blood glucose levels in alloxan induced diabetic mice.

The dry powder of *Acacia nilotica* yielded 5.63% (w/w) aqueous stem bark extracts. At the four therapeutic dose levels (50, 100, 200 and 300 mg/kg body weight) oral administration of the aqueous stem bark extracts of *Acacia nilotica* decreased the blood glucose levels (Table 1, Figure 1). During the 1st hour the percent reductions in the blood glucose levels were 38.72%, 25.84%, 28.83%, and 17.15%, respectively, compared to reference drug glibenclamide which lowered blood glucose levels by 12.52% within the same hour. By the third hour, all the four therapeutic dose levels (50, 100, 200 and 300 mg/kg body weight) lowered blood glucose levels by more than half, that is, 69.01%, 58.32%, 52.44% and 50.67%, respectively, compared to glibenclamide which lowered blood glucose levels by 46.97%

Treatment	Route	Glucose levels at varying times in mmol/dL							
		0 hr	1 hr	2 hr	3 hr	4 hr	6 hr	24 hr	
Control	Oral	5.30 ± 0.28	5.04 ± 0.17	5.02 ± 0.13	5.12 ± 0.23	5.08 ± 0.08	5.18 ± 0.25	4.96 ± 0.21	
	IP	5.12 ± 0.18	5.28 ± 0.22	5.28 ± 0.13	5.12 ± 0.22	5.18 ± 0.08	5.22 ± 0.18	5.22 ± 0.29	
Diabetic control	Oral	24.42 ± 3.18 ^A	25.48 ± 2.79 ^B	26.12 ± 2.35 ^B	26.84 ± 1.92 ^B	27.58 ± 1.45 ^B	28.88 ± 1.11 ^{Ba}	30.08 ± 1.49 ^{Cb}	
	IP	19.84 ± 1.26 ^A	21.48 ± 1.41 ^B	23.08 ± 1.50 ^{Ba}	24.24 ± 1.71 ^{Ba}	25.20 ± 1.36 ^{Ab}	25.74 ± 1.60 ^{Ab}	28.04 ± 1.47 ^{Cc}	
Diabetic/Glib	Oral	23.80 ± 2.72 ^{Ac}	20.82 ± 2.43 ^{Ab}	17.62 ± 3.15 ^{Ab}	12.62 ± 3.68 ^{Aa}	8.68 ± 2.00	5.74 ± 1.06	6.82 ± 1.01	
Diabetic/Insulin	IP	20.88 ± 2.69 ^{Bb}	7.30 ± 0.54 ^a	6.32 ± 0.44	6.08 ± 0.41	5.46 ± 0.47	4.68 ± 0.38	6.98 ± 0.94 ^a	
Extract dose (mg/kg body weight)									
50	Oral	25.88 ± 1.12 ^{Ac}	15.86 ± 2.50 ^{Ab}	11.78 ± 5.33 ^a	8.02 ± 3.14	5.18 ± 1.62	4.68 ± 1.33	6.66 ± 1.09	
	IP	15.42 ± 3.05 ^{Aa}	7.78 ± 1.64	8.22 ± 2.07	6.00 ± 0.98	4.96 ± 1.47	6.02 ± 1.57	8.38 ± 1.54	
100	Oral	22.60 ± 4.50 ^{Ac}	16.76 ± 0.69 ^{Ab}	14.10 ± 2.06 ^{Ab}	9.42 ± 0.89 ^a	5.58 ± 0.94	5.10 ± 1.18	7.58 ± 0.59	
	IP	16.66 ± 3.54 ^{Aa}	9.68 ± 4.66	8.58 ± 3.43	7.42 ± 2.73	6.06 ± 1.57	7.72 ± 3.06	10.46 ± 2.53 ^B	
200	Oral	24.14 ± 4.12 ^{Aa}	17.18 ± 4.68 ^A	12.60 ± 5.07 ^A	11.48 ± 4.71 ^A	11.36 ± 4.72 ^A	8.90 ± 2.17 ^A	12.00 ± 3.32 ^B	
	IP	18.20 ± 1.27 ^{Ac}	13.42 ± 5.10 ^{Ab}	12.40 ± 4.43 ^{Aa}	8.78 ± 2.96 ^A	5.68 ± 0.71	7.12 ± 1.51	9.44 ± 2.44 ^A	
300	Oral	20.88 ± 3.40 ^{Ac}	17.30 ± 1.58 ^{Ab}	14.02 ± 3.41 ^{Aa}	10.30 ± 3.87	8.10 ± 1.55	7.46 ± 2.86	11.32 ± 4.49 ^A	
	IP	15.62 ± 3.87 ^{Aa}	7.76 ± 2.42	7.10 ± 1.88	6.30 ± 1.19	5.50 ± 0.50	5.92 ± 0.71	7.48 ± 1.58	

Values are expressed as Means ± SD for five animals per group. Means within respective columns followed by similar upper case letters are not significantly different at $p \leq 0.05$ by ANOVA and post ANOVA; means within respective rows followed by similar lower case letters are not significantly different at $p \leq 0.05$ by ANOVA and post ANOVA.

Table 1: Effect of oral and intraperitoneal administration of aqueous stem bark extracts *Acacia nilotica* on blood glucose levels in alloxan induced diabetic mice.

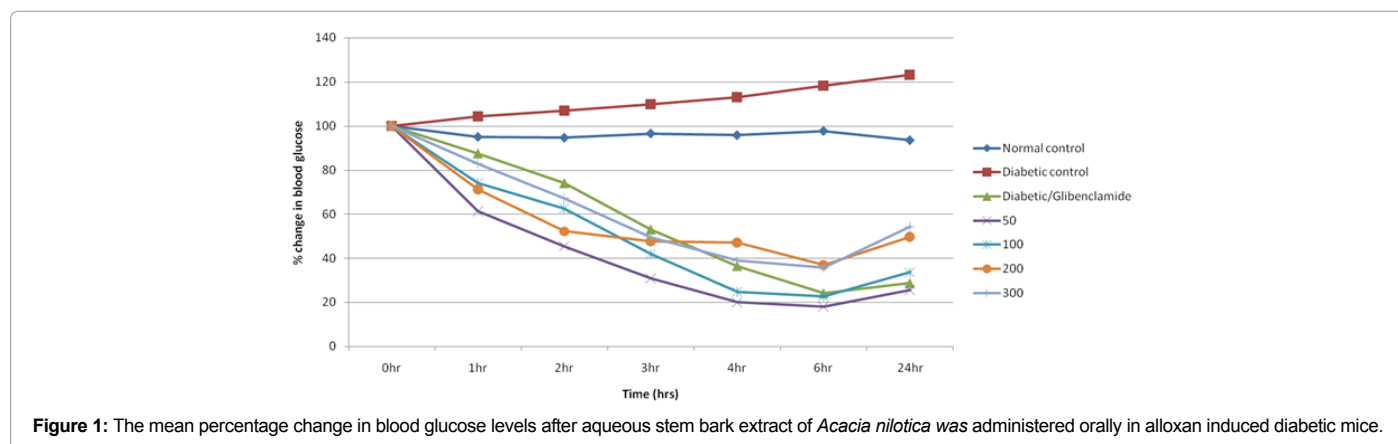


Figure 1: The mean percentage change in blood glucose levels after aqueous stem bark extract of *Acacia nilotica* was administered orally in alloxan induced diabetic mice.

within the same hour. In the 6th hour the percent blood glucose reductions by the four therapeutic doses were 81.92%, 77.43%, 63.13% and 64.27%, respectively, compared to glibenclamide which lowered blood glucose levels by 75.88% within the same hour. After this, a gradual increase was recorded up to the twenty fourth hour.

Intraperitoneal administration of aqueous stem bark extracts at all the four therapeutic doses of *Acacia nilotica* also lowered blood glucose levels from the 1st hour to the 6th hour (Table 1, Figure 2) in a dose independent manner. By the 1st hour the four therapeutic doses of the extract had lowered the blood glucose levels by 49.55%, 41.60%, 26.26%, and 50.32% respectively, compared to insulin which had lowered blood sugar levels by 65.04% within the same hour.

In the 6th hour, the percent blood glucose reductions by the four therapeutic doses were 60.96, 53.66, 60.88 and 62.10%, respectively, compared to insulin which had lowered blood sugar levels by 77.59% within the same hour. After this, a gradual increase was recorded up to the twenty fourth hour.

Effect of oral and intraperitoneal administration of aqueous stem bark extracts of *Acacia nilotica* at 1 g/kg body weight to mice for one month on the weekly change in body weight and percent organ to body weight.

Results are shown in Tables 2 and 3. Oral administration 1 g/kg body weight of aqueous stem bark extracts of *Acacia nilotica* to mice for one month did not significantly alter the weekly change in body weight and percent organ to body weights of all the studied organs relative to those of the normal control mice (Table 2 and 3).

Intraperitoneal administration of the aqueous stem bark extracts of *Acacia nilotica* at 1 g/kg body weight to mice for one month significantly decreased the weekly change in body weight and the percent organ to body weight of testes while not significantly altering the percent organ to body weight of the other studied organs relative to those of the normal control mice (Table 2 and 3).

Effect of oral and intraperitoneal administration of 1 g/kg body weight of aqueous stem bark extracts of *Acacia nilotica* for one month on some hematological parameters in mice.

Table 4 shows the effect of oral and intraperitoneal administration of stem bark extracts of *Acacia nilotica* for one month on some hematological parameters in mice. Oral administration of 1 g/kg body weight of aqueous stem bark extracts of *Acacia nilotica* to mice for one month significantly decreased the level of PLT but did not significantly

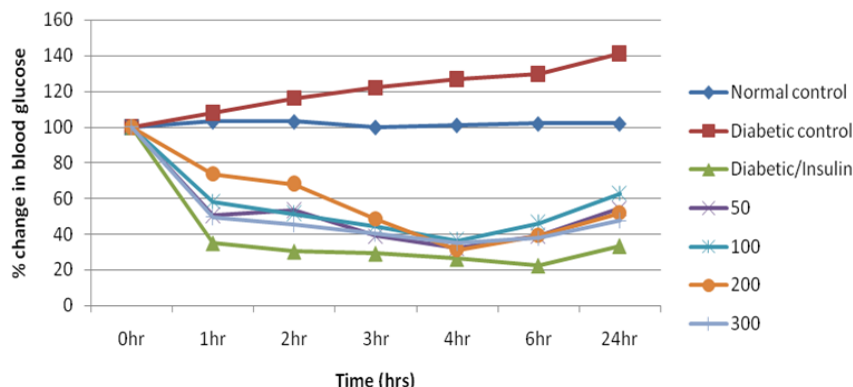


Figure 2: The mean percentage change in blood glucose levels after aqueous stem bark extract of *Acacia nilotica* was administered intraperitoneally in alloxan induced diabetic mice.

Treatment	Average weekly mice weight (g)					Δ weight/week
	0	1	2	3	4	
Control Oral	14.0 ± 1.4	15.8 ± 1.5	16.9 ± 0.7	19.0 ± 0.7	20.8 ± 1.1	1.705 ± 0.522
<i>Acacia nilotica</i> Oral	13.8 ± 0.4	14.8 ± 0.7	15.1 ± 1.8	16.2 ± 1.8*	17.6 ± 2.0*	0.940 ± 0.554
Control IP	15.3 ± 2.5	16.7 ± 3.1	18.9 ± 3.3	21.5 ± 2.8	24.0 ± 2.4	2.155 ± 0.089
<i>Acacia nilotica</i> IP	21.2 ± 2.4*	22.0 ± 1.9*	23.0 ± 1.7*	23.6 ± 1.6	24.5 ± 1.7	0.835 ± 0.212*

Average weekly mice weight and average weekly change in mice body weight are measured in g. Results are expressed as Mean ± Standard Deviation (SD) for five animals for each parameter; *p<0.05 is considered statistically significant when the mean of the experiment group is compared to its relevant control group by T-test.

Table 2: The effects of oral and intraperitoneal administration of 1 g/kg body weight of aqueous stem bark extracts of *Acacia nilotica* in mice for one month on average weekly body weight and weekly change in body weight.

Treatment	Percent organ to body weight (g)						
	Liver	Brain	Kidney	Lungs	Spleen	Heart	Testes
Control Oral	7.27 ± 1.12	2.74 ± 0.27	1.78 ± 0.24	1.79 ± 0.21	1.12 ± 0.32	0.53 ± 0.09	0.93 ± 0.16
<i>Acacia nilotica</i> Oral	7.93 ± 1.11	3.30 ± 0.61	1.89 ± 0.18	1.85 ± 0.44	1.14 ± 0.21	0.56 ± 0.15	0.77 ± 0.22
Control IP	5.01 ± 1.26	1.75 ± 0.31	1.39 ± 0.42	1.38 ± 0.54	0.61 ± 0.13	0.51 ± 0.21	0.91 ± 0.22
<i>Acacia nilotica</i> IP	4.75 ± 0.48	1.94 ± 0.36	1.19 ± 0.16	1.10 ± 0.24	0.54 ± 0.08	0.32 ± 0.04	0.59 ± 0.06*

Results are expressed as Mean ± Standard Deviation (SD) for five animals for each parameter; *p<0.05 is considered statistically significant when the mean of the experiment group is compared to its relevant control group by T-test.

Table 3: The effects of oral and intraperitoneal administration of aqueous stem bark extracts of *Acacia nilotica* at 1g/kg body weight in mice for one month on the percent organ to body weight.

Treatment	Hematological parameters						
	RBC (x10 ⁶ /μL)	Hb (g/dL)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT (x10 ³ /μL)
Control Oral	7.61 ± 0.74	9.58 ± 0.73	32.74 ± 2.49	43.12 ± 2.84	12.64 ± 0.96	29.30 ± 0.54	607.40 ± 116.07
<i>Acacia nilotica</i> Oral	8.45 ± 1.32	10.24 ± 1.57	36.00 ± 5.68	42.60 ± 2.08	12.42 ± 0.93	29.12 ± 1.06	422.68 ± 69.92*
Control IP	6.38 ± 0.67	8.98 ± 0.80	28.66 ± 2.64	45.02 ± 1.44	14.10 ± 0.51	31.36 ± 0.66	352.80 ± 73.32
<i>Acacia nilotica</i> IP	5.32 ± 1.27	7.50 ± 1.88	24.98 ± 4.97	47.52 ± 3.44	14.06 ± 0.48	29.74 ± 2.62	341.00 ± 39.64

Results are expressed as Mean ± standard deviation (SD) for five animals in each treatment; *p<0.05 is considered significant when the mean of the control animals is significantly different from that of the extract treated animals by T-Test.

Table 4: The effects of oral and intraperitoneal administration of 1 g/kg body weight of aqueous stem bark extracts of *Acacia nilotica* in mice for one month on some hematological parameters.

change the levels of RBC, Hb, PCV, MCV, MCH, and MCHC relative to those of the normal control mice. In addition, intraperitoneal administration of the same dose of aqueous stem bark extracts of *Acacia nilotica* to mice for one month did not significantly change the levels of all the measured hematological parameters relative to those of the normal control mice.

Effect of oral and intraperitoneal administration of aqueous stem bark extracts of *Acacia nilotica* at 1 g/kg body weight to mice for one month on differential white blood cell count.

Results are shown in Table 5. Oral and intraperitoneal administration of aqueous stem bark extracts of *Acacia nilotica*, at 1 g/kg body weight to mice for one month did not cause significant changes to the differential white blood cell count.

Effects of oral and intraperitoneal administration of 1 g/kg body weight of aqueous stem bark extracts of *Acacia nilotica* in mice for one month on biochemical parameters

Oral administration of 1 g/kg body weight of aqueous stem bark extracts of *Acacia nilotica* caused a significant increase in the levels of

GGT, CK, and T-BIL while decreasing the levels of ALT, AST, AMY, and ALP relative to that of the normal control mice; however, there was no significant alteration in the levels of Urea, LDH, D-BIL, and Creatinine by the same extract dose compared to that of the respective control group (Table 6 and 7). Intraperitoneal administration of the same dose of aqueous stem bark extracts of *Acacia nilotica* significantly decreased the level of AST while not altering the levels of Urea, ALT, GGT, CK, LDH AMY, ALP, T-BIL, D-BIL, and Creatinine compared to that of the respective normal control group (Table 6 and 7).

Histopathology

Oral and Intraperitoneal administration of 1 g/kg body weight of aqueous stem bark extracts of *Acacia nilotica* did not cause any significant histopathological lesion on the liver, brain, kidney, lung, spleen, heart and testes when compared to those of the normal control mice.

Qualitative and quantitative phytochemical screening

As Table 8 shows, Phytochemical screening of aqueous extracts of *Acacia nilotica* indicated the presence of phenols, alkaloids, flavonoids, tannins, saponins, in the aqueous stem bark extract of *Acacia nilotica*.

Mineral Elements Analysis

Table 9 shows the mineral composition of the aqueous stem bark extract of *Acacia nilotica*. Sodium (Na), chlorine (Cl), potassium (K), Calcium (Ca), Titanium (Ti), Vanadium (V), chromium (Cr), manganese (Mn), iron (Fe), Copper (Cu), Zinc (Zn), Arsenic (As), Nickel (Ni) Lead (Pb) and Cadmium (Cd) were present in the aqueous stem bark extract of *Acacia nilotica*. The levels of these measured minerals and trace element were all below the recommended daily allowance.

Discussion

The present work was undertaken to investigate *in vivo* the hypoglycemic activity and safety of the aqueous stem bark extracts of *Acacia nilotica* in alloxan-induced diabetic mice and normal mice respectively. The alloxan-induced diabetic mice had an average of 391% increase in blood glucose levels compared to normal control group.

The study demonstrated that both oral and intraperitoneal administration of the aqueous stem bark extracts of *Acacia nilotica* had shown hypoglycemic activity at the four dose levels tested (50 mg/kg body weight 100 mg/kg body weight, 200 mg/kg body weight, and 300 mg/kg body weight).

These findings agrees with the results obtained by [16] who reported the blood glucose lowering effect of the aqueous and ethylacetate leaf and stem bark extracts of *pappea capensis* in alloxan induced diabetic mice. In addition [30] demonstrated the hypoglycemic activity of aqueous extracts of five Kenyan medicinal plants in alloxan induced diabetes mice.

The hypoglycemic properties of this plant extract could result from the alkaloids, saponins, total phenols, flavonoids, and tannins which are present in this plant extract. For instance alkaloids collected from leaves of *Acanthus montanus* intraperitoneally administered at doses of 100, 200 and 400 mg/kg body weight showed hypoglycemic action in alloxan-induced diabetic rats [31]. In addition alkaloids of *Ephedra distachya* herbs and l-ephedrine have shown antihyperglycemic effect in diabetic mice due to regeneration and restoration of atrophied pancreatic islets that induces the secretion of insulin [32].

Saponins have also been reported to be antidiabetic. Saponins are glycosides of triterpenes, steroids or alkaloids. Previous studies have demonstrated the hypoglycemic activity of triterpenoid glycosides [33,34]. For instance ginseng and its saponins have been shown to lower blood glucose in alloxan-treated, genetically diabetic, and normal mice [35]. In elderly patients with hyperglycemia, saponins were shown to reduce serum glucose [36].

Flavonoids act on various molecular targets and regulate different signaling pathways in pancreatic β -cells, hepatocytes, adipocytes and skeletal myofibers [37]. Several studies have demonstrated the hypoglycemic action of flavonoids using different experimental methods, for instance [38] reported that total flavonoids from *Litsea coreana* leaves showed a significant increase in insulin sensitivity, serum High Density Lipoprotein- cholesterol HDL-C level, and also decreased the expression of protein tyrosine phosphatase 1B (PTP1B) in diabetic rat liver.

Treatment	White blood cell and differential white blood cell count					
	WBC (x10 ³ / μ L)	Neutrophils (x10 ³ / μ L)	Lymphocytes (x10 ³ / μ L)	Eosinophils (x10 ³ / μ L)	Monocytes (x10 ³ / μ L)	Basophils (x10 ³ / μ L)
Control Oral	14.34 \pm 3.48	4.46 \pm 1.26	7.27 \pm 1.68	1.35 \pm 0.41	1.15 \pm 0.21	0.09 \pm 0.08
<i>Acacia nilotica</i> Oral	13.53 \pm 5.98	3.70 \pm 1.68	7.45 \pm 3.22	1.27 \pm 0.55	1.06 \pm 0.55	0.04 \pm 0.09
Control IP	6.87 \pm 0.71	2.18 \pm 0.25	3.51 \pm 0.33	0.59 \pm 0.14	0.54 \pm 0.13	0.04 \pm 0.04
<i>Acacia nilotica</i> IP	6.74 \pm 2.19	1.82 \pm 0.65	3.74 \pm 1.22	0.61 \pm 0.21	0.53 \pm 0.14	0.03 \pm 0.05

Results are expressed as Mean \pm Standard Deviation (SD) for five animals in each treatment; *p<0.05 is considered significant when the mean of the control animals is significantly different from that of the extract treated animals by T-Test.

Table 5: The effects of oral and intraperitoneal administration of 1g/kg body weight of aqueous stem bark extracts of *Acacia nilotica* in mice for one month on differential white blood cell count (WBC).

Treatment	Enzyme Activities						
	ALT (U/L)	AST (U/L)	GGT (U/L)	LDH (U/L)	CK (U/L)	AMY (U/L)	ALP (U/L)
Control Oral	132.6 \pm 20.6	692.3 \pm 51.4	1.8 \pm 0.2	1972.9 \pm 158.7	953.4 \pm 74.7	2940.2 \pm 174.7	103.2 \pm 9.1
<i>Acacia nilotica</i> Oral	86.2 \pm 3.7*	488.2 \pm 68.7*	3.1 \pm 0.6*	1889.8 \pm 241.0	2150.5 \pm 257.1*	2115.6 \pm 230.7*	82.3 \pm 6.9*
Control IP	80.3 \pm 7.0	523.2 \pm 94.7	2.0 \pm 1.0	2137.2 \pm 159.4	351.0 \pm 59.1	1676.4 \pm 230.2	46.6 \pm 10.4
<i>Acacia nilotica</i> IP	89.4 \pm 6.4	368.2 \pm 77.1*	1.8 \pm 1.3	1935.2 \pm 238.4	392.8 \pm 46.3	1513.4 \pm 189.5	46.0 \pm 10.9

Table 6: The effects of oral and intraperitoneal administration of 1 g/kg body weight of aqueous stem bark extracts of *Acacia nilotica* in mice for one month on enzyme activities.

Results are expressed as Mean \pm Standard Deviation (SD) for five animals in each treatment; *p<0.05 is considered significant when the mean of the control animals is significantly different from that of the extract treated animals by T-Test.

Treatment	Metabolite Levels			
	UREA (mmol/L)	CREAT (μmol/L)	T-BIL (μmol/L)	D-BIL (μmol/L)
Control Oral	9.6 ± 0.7	75.0 ± 8.5	0.8 ± 0.1	0.4 ± 0.1
<i>Acacia nilotica</i> Oral	8.7 ± 1.0	75.8 ± 8.5	1.2 ± 0.3*	0.5 ± 0.1
Control IP	7.2 ± 0.8	43.2 ± 6.1	4.5 ± 1.2	2.6 ± 0.7
<i>Acacia nilotica</i> IP	8.0 ± 0.7	37.0 ± 6.9	5.8 ± 2.1	3.4 ± 1.1

Table 7: The effects of oral and intraperitoneal administration of 1 g/kg body weight of aqueous stem bark extracts of *Acacia nilotica* in mice for one month on the levels of selected metabolites.

Results are expressed as Mean ± Standard Deviation (SD) for five animals in each treatment; *p<0.05 is considered significant when the mean of the control animals is significantly different from that of the extract treated animals by T-Test.

Phytochemicals Present	Quantities (mg/g)
Tannins	0.4547 ± 0.058
Total Phenols	3.5410 ± 0.220
Flavonoids	3.6141 ± 0.226
Saponins	105.600 ± 9.994
Alkaloids	39.180 ± 1.782

Results are expressed as Mean ± Standard Deviation (SD). Tannins and Total Phenols are expressed as mg/g gallic acid equivalent (GAE), flavonoids are expressed as mg/g quercetin equivalent (QE), alkaloids and saponins are in mg/g.

Table 8: Quantitative analysis of the phytochemical composition of the aqueous stem bark extracts of *Acacia nilotica*.

Element	Mineral extract levels (μg/g)	Amounts (μg) administered to each mouse	RDA for mice (μg/day)
Na*	2448.2 ± 275.2	61.205	5 x 10 ⁵ (178.6)
Mg*	<200	<5	4.2 x 10 ⁵ (150)
Cl*	199.3 ± 4.2	4.9825	7.5 x 10 ⁵ (267.9)
K*	416.5 ± 6.5	10.4125	3.5 x 10 ⁶ (1250)
Ca*	675.5 ± 9.7	16.8875	1.0 x 10 ⁶ (357.1)
Ti	11.4 ± 0.3	0.285	
V	5.8 ± 0.2	0.145	<1.8 x 10 ³ (<0.64)
Cr*	0.009 ± 0.003	0.000225	3.5 x 10 (12.5)
Mn*	2.0 ± 0.1	0.05	2.3 x 10 ³ (0.82)
Fe*	21.52 ± 0.39	0.538	8.0 x 10 ³ (2.9)
Ni	0.06 ± 0.03	0.0015	<1 x 10 ³ (<0.36)
Cu*	0.32 ± 0.03	0.008	1.5 x 10 ³ (0.54)
Zn*	0.40 ± 0.03	0.01	1.1 x 10 ⁴ (3.9)
As	0.12 ± 0.02	0.003	
Se	<0.030	<0.00075	3.5 x 10 (0.0125)
Hg	<0.050	<0.00125	
Pb	0.33 ± 0.03	0.00825	
Cd	2 ± 0.8	0.05	

Results on the concentration of each mineral are expressed as μg/g of dry powder of the aqueous stem bark extracts of *Acacia nilotica* of three determinations and are in the second column; the amount of each mineral administered in μg based on its concentration in the plant's extract is in the third column. This is compared with the (RDA) shown in the last column. This is expressed per the average weight of each mouse. *Recommended daily allowance estimated from that of human beings stated in [58].

Table 9: Mineral levels and amount given to each mouse from the aqueous stem bark extracts of *Acacia nilotica*.

There are two types of tannins; condensed tannins and hydrolysable tannins. Hydrolysable tannins consist of 2 main groups, Tannic acid (TA), also called gallotannins, and ellagitannins [39]. It was found that condensed tannins extracted from selected Kenyan foods exhibited antidiabetic activity through inhibition of α-amylase and α-glucosidase enzymes [40]. In addition commercially available tannic acid were found to induce phosphorylation of the insulin receptor (IR) and cause translocation of glucose transporter 4 (GLUT 4) [39].

Apart from these phytochemical components, the hypoglycemic action of the plant of this study could be related to the presence of trace elements like: chromium (Cr), zinc (Zn), iron (Fe), magnesium (Mg), manganese (Mn), selenium (Se), vanadium (V) Calcium (Ca) and Potassium (K). These elements possess antidiabetic activity. For instance the trivalent Cr is a potentiator of insulin action [41]. It is a part of glucose tolerance factor (GTF), a biologically active substance manufactured in the body that regulates glucose biotransformation and increases the number of insulin receptors, enhances receptor binding, and potentiates insulin action [42]. It was found that experimental chromium deficiency leads to impaired glucose tolerance, which is improved by chromium supplementations [43,44].

Zinc is virtually involved in all aspects of insulin metabolism: synthesis, secretion and utilization [42]. It is a powerful guardian against viral infections, and play role in protecting β-cells from destruction. Type 1 diabetics are often zinc deficient, and zinc supplements have been shown to lower blood sugar levels in some of these cases [45]. Magnesium modulates glucose transport across cell membranes and is a cofactor in various enzymatic pathways involved in glucose oxidation [43]. Four weeks dietary magnesium supplementation has been reported to improve insulin secretory capacity [46].

A deficiency of manganese is common amongst diabetics [45]. Experimental evidence suggests that manganese (Mn) deficiency in guinea pigs can cause impaired glucose utilization and Mn supplementation can reverse glucose intolerance induced by Mn deficiency [47]. Selenium has been shown to mediate a number of insulin-like actions both *in vivo* and *in vitro*. These insulin-like actions include stimulating glucose uptake and regulating metabolic processes such as glycolysis, gluconeogenesis, fatty acid synthesis and the pentose phosphate pathway [48]. Vanadium was used for the control of blood sugar before the discovery of insulin [42]. In animal models, vanadium has been shown to facilitate glucose uptake and metabolism, and enhance insulin sensitivity [44,49,50].

Calcium improves insulin sensitivity in some type 2 diabetic populations [51]. Potassium supplementation yields improved insulin sensitivity, responsiveness and secretion; insulin administration induces a loss of potassium; and a high potassium intake reduces the risk of heart disease, atherosclerosis, and cancer [52,53]. Iron influences glucose metabolism and reciprocally, iron influences insulin action. Iron interferes with insulin inhibition of glucose production by the liver [54].

The intraperitoneal administration of the aqueous stem bark extracts of *Acacia nilotica* caused decrease in growth rate. This decrease in growth rate may have been caused by the presence of alkaloids, saponins, flavanoids, and tannins. Alkaloids such as ρ-octopamine and synephrines may reduce body weight by exerting adrenergic agonist activity [55]. Synephrines increase energy expenditure (EE) (resting energy expenditure [70%], thermic effect of feeding [10%], and energy expenditure of physical activity [20%]) and decrease food intake in

addition to decreasing gastric motility (slows gastric emptying and intestinal transit) and indirectly producing increased feeling of satiety and a decreased appetite [55].

The flavanoid, chlorogenic acid reduces body weight by inducing reduction in body fat by reducing the absorption of glucose (energy source) leading to an increase in the consumption of fat reserves. Catechins (flavanoids) were shown to induce reduction of body fat by inhibiting small intestine micelle formation and inhibiting α -glucosidase activity leading to decreased carbohydrate absorption [55].

The reduced growth rates of the experimental mice intraperitoneally administered with 1g/kg body weight of the extract relative to the control mice correlates with the reduced percent organ to body weight of testes (*Acacia nilotica* intraperitoneally administered). Normalization of organ weight to body weight helps eliminate variations due to body weight differences as some apparent difference in organ weight might have been the result of differences in body weight which are unrelated to the treatment [56].

The investigated hematological parameters in this study are useful in the assessment of the toxic potentials of the plant extracts in mammals and human beings. They provide information about the status of bone marrow activity and hemolysis [57,58]. Among the hematological parameters measured in this study only platelet levels were significantly reduced by the oral administration of the aqueous stem bark extracts of *Acacia nilotica* at 1 g/kg body weight dose level.

The abnormalities in the levels γ -glutamyltransferase, creatine kinase, alanine aminotransferase, aspartate aminotransferase, α -amylase, alkaline phosphatase and total bilirubin caused by the administration of the aqueous stem bark extracts of *Acacia nilotica* at 1 g/kg body weight dose level may be due to tissue injury in organs like liver, heart, kidney, skeletal muscles and pancreas e.t.c. caused by the constituents of this plant.

The observation of a general reduction in weekly weight gain, reduction in percent organ to body weight of tests together with the biochemical and hematological abnormalities without obvious histopathological lesions displays the poor diagnostic capacity of histopathology in sub-clinical situations.

Conclusion

The aqueous stem bark extracts of *Acacia nilotica* had antidiabetic activity. The intraperitoneal route was more effective in reducing the blood glucose levels than the oral route. The aqueous stem bark extracts of *Acacia nilotica* at high dose of 1 g/kg body weights which is far from the therapeutic dose tends to cause subclinical toxicological effects. This was well demonstrated in the body and organ weight changes, hematological, and biochemical parameters. The antidiabetic and toxic action of the studied plant may have resulted from its phytochemical and mineral constituents.

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