

# Blood Glucose Lowering Effect and Safety of the Aqueous Leaf Extracts of *Zanha africana*

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## Abstract

*Zanha africana* has been used traditionally to manage many diseases including diabetes, however, its antidiabetic activity and safety is not well evaluated. The aim of this study was to determine *in vivo* hypoglycemic activity and safety of the aqueous leaf extracts of this plant in male Swiss white albino mice. The antidiabetic activity was screened in alloxan induced diabetic mice using oral and intraperitoneal routes. The safety of the 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 weights, hematological and biochemical parameters. Mineral composition was estimated using total reflection X-ray fluorescence system and atomic absorption spectrometry. Phytochemical composition was assessed using standard procedures. The extract showed hypoglycemic activity at dose levels of 50, 100, 200, 300 mg/kg body weight. Administration of 1 g/kg body weight of the extract decreased the body weight gain using both routes. Intraperitoneal administration of the same dose increased the organ to body weight percentages of liver, brain and kidney, and elevated white blood cell count, lymphocyte count, and levels of  $\gamma$ -glutamyl transpeptidase, total bilirubin and direct bilirubin and decreased levels of aspartate aminotransferase and creatinine. Increase in levels of mean corpuscular hemoglobin,  $\gamma$ -glutamyl transpeptidase, lactate dehydrogenase and creatine kinase, and decrease in levels of platelets, alanine transaminase, aspartate aminotransferase, urea, creatinine, total bilirubin and direct bilirubin was recorded in mice orally administered with 1 g/kg body weight of the extract. The extract contained tannins, phenols, flavonoids, saponins, and alkaloids. Sodium, Chlorine, Potassium, Calcium, Titanium, Vanadium, Chromium, Manganese, Iron, Copper, Zinc, Arsenic, Cadmium, Magnesium, Nickel and Lead were present in the extracts at levels below the recommended daily allowance. The observed hypoglycemic activity and slight toxicity could be associated with the phytochemicals and mineral/ trace elements present in this extract.

**Keywords:** *Zanha africana*; Diabetes mellitus; Biochemical parameters; Hematological parameters; Hypoglycemic effect; Phytochemicals

## Introduction

Diabetes mellitus is a metabolic disorder with increasing rates of incidence and mortality [1]. According to the International Diabetes Federation (IDF) the numbers of people suffering from diabetes are over 382 million worldwide and this number is expected to increase to over 592 million in less than 25 years [2]. The disease is characterized by hyperglycemia resulting from either defect in insulin secretion or insulin action or both [3]. Insulin is a hormone manufactured by the  $\beta$ -cells of the pancreas, which is required to uptake and utilize glucose as an energy source [4].

Lack of insulin or insulin resistance prevents efficient glucose uptake by most body cells except brain cells. This results in increased blood glucose levels, reduced cell utilization of glucose and increased utilization of fats and proteins as energy sources [5]. Acute, life-threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the non-ketotic hyperosmolar syndrome. The long-term microvascular and macrovascular complications of the disease include; neuropathy (nerve damage), nephropathy (renal disease), vision disorders, cardiovascular vascular disorders, stroke and peripheral vascular diseases which can lead to ulcers, gangrene and amputation [3].

Treatment of type I diabetes requires administration of exogenous insulin so as the patient will have normal carbohydrate, protein and fat metabolism [5]. However weight gain and hypoglycemia are common side effects of insulin therapy [6]. For type II patients treatment options begin with diet and life style modifications but as disease progresses often oral hypoglycemic agents or insulin or both are required [7].

Five classes of oral agents are approved for the treatment of diabetes. Although initial response may be good, oral hypoglycemic drugs may lose their effectiveness in a significant percentage of patients. The oral hypoglycemic drugs include; sulfonylurea, biguanide,  $\alpha$ -glucosidase inhibitor, thiazolidinedione, and meglitinide. These drugs have various side effects; sulfonylureas cause weight gain, biguanide cause weakness, fatigue, and lactic acidosis,  $\alpha$ -glucosidase inhibitor may cause diarrhea while thiazolidinediones may increase LDL-cholesterol level [8].

There is a growing interest in herbal remedies to avoid the side effects associated with the conventional antidiabetic drugs [9]. The hypoglycemic action of a notable number of medicinal plants has been confirmed in animal models and non-insulin-dependent diabetic patients, and various hypoglycemic compounds have been identified. Traditional antidiabetic plants might provide a useful source of new oral hypoglycemic compounds for development as pharmaceutical entities, or as simple dietary adjuncts to existing therapies [10].

*Zanha africana* is a plant remedy used by traditional health practitioners for treatment of many diseases such as diarrhea, typhoid

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fever, pneumonia, scabies, nose bleeding and to prevent and stop bleeding for women [11].

The antibacterial activity of both water and organic extracts of this plant has been demonstrated with the methanol extracts exhibiting the greatest antibacterial activity [12]. The plant has considerable antifungal activity against candida species [13]. Moreover the crude extracts of this plant caused substantial growth inhibition for *Trypanosoma brucei* [14].

## Materials and Methods

### Study site

This study was undertaken at the Department of Biochemistry and Biotechnology, School of Pure and Applied Sciences, Kenyatta University from December 2012 to August 2014. Kenyatta University is 23 km from Nairobi off Thika Road.

### Collection of the plant materials and preparation of the aqueous extract

The plant used in this study was collected from its natural habitat in Machakos County, Kenya. An acknowledged authority in taxonomy authenticated the botanical identity of the plant. The collected leaves of *Zanha africana* were left to dry under shed at room temperature for 1 month, and then ground when completely dry using an electric mill. Each one hundred grams of the powdered plant material was extracted in 1 liter distilled 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 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 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) [15].

Forty-eight hours after alloxan administration, blood glucose level was measured using a glucometer. Mice with blood glucose levels above 200 mg/dL were considered diabetic and used in this study. Prior to initiation of this experiment, the animals were fasted for 8-12 hours [16] but allowed free access to water until the end of this experiment.

### Experimental design

The experimental design used in this study is shown in Table 1a and Table 1b.

**Blood sampling and glucose determination:** Blood sampling was done by sterilizing the tail with 70% 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 (Hypogaurd, 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 administered daily for 28 days with the extract at 1 g/kg body weight in 0.1 ml physiological saline. During this period, mice were allowed free

Group	Description	Treatment	Number of Mice
1	Normal control	Orally administered with 0.1 ml physiological saline	5
2	Diabetic control	Orally administered with 0.1 ml physiological saline	5
3	Diabetic reference	Orally administered with 0.075 mg glibenclamide (3 mg/kg body weight) in 0.1 ml physiological saline	5
4	Diabetic	Orally administered with 1.25 mg extract (50 mg/kg body weight) in 0.1 ml physiological saline	5
5	Diabetic	Orally administered with 2.5 mg extract (100 mg/kg body weight) in 0.1 ml physiological saline	5
6	Diabetic	Orally administered with 5 mg extract (200 mg/kg body weight) in 0.1 ml physiological saline	5
7	Diabetic	Orally administered with 7.5 mg extract (300 mg/kg body weight) in 0.1 ml physiological saline	5

The experimental mice were randomly divided into seven groups of five animals each. Either 0.1 ml of physiological saline, glibenclamide or the plant extract solution was administered to each experimental mouse orally.

Table 1a: Experimental design for oral administration in mice.

Group	Description	Treatment	Number of Mice
1	Normal control	Intraperitoneally administered with 0.1 ml physiological saline	5
2	Diabetic control	Intraperitoneally administered with 0.1 ml physiological saline	5
3	Diabetic reference	Intraperitoneally administered with 0.025 Insulin units (1 IU/kg body weight) in 0.1ml physiological saline	5
4	Diabetic	Intraperitoneally administered with 1.25 mg extract (50 mg/kg body weight) in 0.1 ml physiological saline	5
5	Diabetic	Intraperitoneally administered with 2.5 mg extract (100 mg/kg body weight) in 0.1 ml physiological saline	5
6	Diabetic	Intraperitoneally administered with 5 mg extract (200 mg/kg body weight) in 0.1 ml physiological saline	5
7	Diabetic	Intraperitoneally administered with 7.5 mg extract (300 mg/kg body weight) in 0.1 ml physiological saline	5

The experimental mice were randomly divided into seven groups of five animals each. Either 0.1 ml of physiological saline, insulin, or the plant extract solution was administered to each experimental mouse intraperitoneally.

Table 1b: Experimental design for intraperitoneal administration.

access to mice pellet and water and observed for any signs of general illness, change in behavior 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 28<sup>th</sup> 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 anesthesia 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 and weighed.

**Determination of hematological parameters:** Blood parameters and indices were determined using standard protocols [17]. 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 [17]. Air-dried thin blood films stained with giemsa stain were examined microscopically using magnification of 400 for differential WBC counts.

**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 ( $\gamma$ -GT), lactate dehydrogenase (LDH), creatine kinase (CK),  $\alpha$ -amylase ( $\alpha$ -AMYL), total bilirubin (T-BIL), direct bilirubin (D-BIL), urea and creatinine. All reagents for the machine were commercially prepared 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.

**Qualitative analysis on phytochemical constituents:** The extract was screened for the presence of five major classes of phytochemicals using the recommended procedures. Alkaloids [18], Saponins [19], Flavonoids [20], Phenols [19], and Tanins [20].

**Quantitative analysis on phytochemical constituents:** The phytochemicals present were quantified using standard procedures. Alkaloids [21] Saponins [22] Flavonoids [23] Phenols [24] and Tannins [25].

**Mineral elements analysis:** Mineral composition of the plant extract 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), 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 [26]. Atomic absorption spectrometry (AAS) was used for the analysis of Magnesium, Chromium and Cadmium [27]. All the analysis were 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 extract 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 aqueous leaf extracts of *Zanha africana* on blood glucose levels in alloxan induced diabetic mice

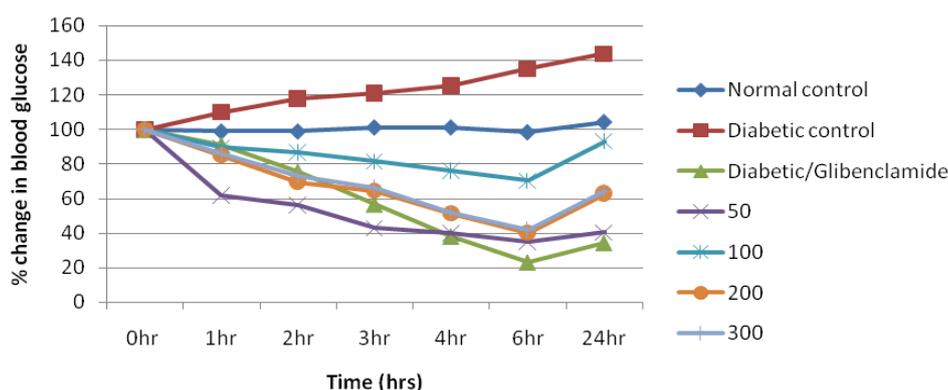
The dry powder of *Zanha africana* yielded 3.45% (w/w) aqueous leaf extract. Oral administration of aqueous leaf extracts of *Zanha africana* at the four therapeutic dose levels (50, 100, 200 and 300mg/kg body weight) decreased the blood glucose levels from the 1<sup>st</sup> hour to the 6<sup>th</sup> hour in a dose independent manner. Thereafter, there was a gradual increase up to the 24<sup>th</sup> hour (Table 2, Figure 1). During the 1<sup>st</sup> hour, the percent reductions in the blood glucose levels by the four aqueous leaf extract doses were 37.87%, 10.38%, 14.76%, and 14.06%, respectively, compared to reference drug glibenclamide which lowered blood glucose levels by 8.87% within the same hour. In this hour, the four tested dose levels did not lower blood glucose levels to normal. In the 6<sup>th</sup> hour, the percent blood glucose reductions by the four aqueous leaf extract doses were 64.89%, 29.77%, 59.78% and 58.19% respectively, compared to glibenclamide which lowered blood glucose levels by 77.13% within the same hour. In this hour, the dose level 50 mg/kg body weight decreased blood glucose levels to normal and was effective as glibenclamide. However the dose levels 100 mg/kg body weight, 200 mg/kg body weight and 300 mg/kg body weight did not lower blood glucose levels to normal and were not effective as glibenclamide. After this, a gradual increase was recorded up to the twenty fourth hour.

Intraperitoneal administration of aqueous leaf extract at all four dose levels (50, 100, 200 and 300 mg/kg body weight) of *Zanha africana* also lowered blood glucose levels from the 1<sup>st</sup> hour to the 6<sup>th</sup> hour (Table 2, Figure 2) in a dose independent manner. By the 1<sup>st</sup> hour, the four extract doses had lowered the blood glucose levels by 6.91%, 10.70%, 13.25%, and 13.70%, respectively, compared to insulin which had lowered blood sugar levels by 68.40% within the same hour. In this hour, all tested dose levels did not lower blood glucose levels to normal. By the 6<sup>th</sup> hour, all the four dose levels (50, 100, 200 and 300 mg/kg body weight) of *Zanha africana* lowered blood glucose levels by more than half, that is, 70.22%, 55.49%, 57.65% and 63.56%, respectively, compared to insulin which had lowered blood glucose levels by 76.30% within the same hour. In this hour, the dose level 50 mg/kg body

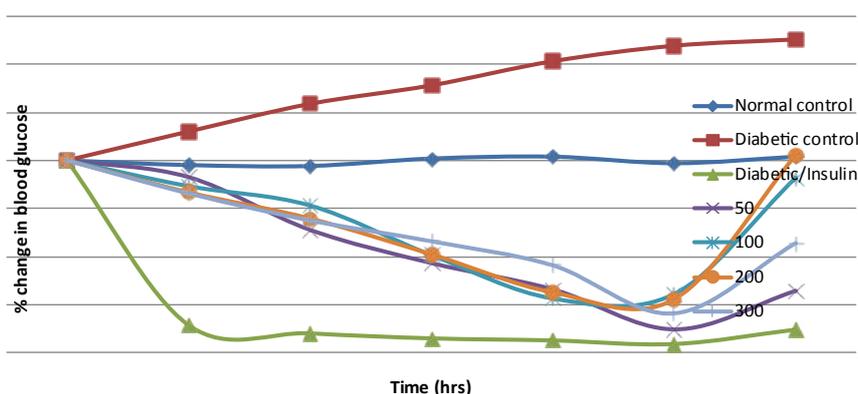
Treatment	Route	Glucose levels at varying times in mmole/L						
		0 hr	1 hr	2 hr	3 hr	4 hr	6 hr	24 hr
Normal control	Oral	5.12 ± 0.15	5.06 ± 0.21	5.06 ± 0.13	5.18 ± 0.13	5.18 ± 0.13	5.04 ± 0.11	5.34 ± 0.22
	IP	5.18 ± 0.13	5.08 ± 0.13	5.06 ± 0.15	5.22 ± 0.13	5.26 ± 0.34	5.12 ± 0.13	5.26 ± 0.13
Diabetic control	Oral	20.64 ± 1.13 <sup>A</sup>	22.72 ± 1.09 <sup>Ba</sup>	24.32 ± 1.13 <sup>Ca</sup>	24.94 ± 1.07 <sup>Cb</sup>	25.88 ± 0.97 <sup>Cb</sup>	27.84 ± 0.87 <sup>Cc</sup>	29.66 ± 0.76 <sup>Cd</sup>
	IP	20.52 ± 1.68 <sup>A</sup>	22.98 ± 2.38 <sup>B</sup>	25.34 ± 1.80 <sup>Ba</sup>	26.92 ± 1.57 <sup>Cb</sup>	28.98 ± 1.44 <sup>Cc</sup>	30.30 ± 0.73 <sup>Cd</sup>	30.84 ± 0.39 <sup>Cd</sup>
Diabetic/Gliben	Oral	21.86 ± 1.98 <sup>Ad</sup>	19.92 ± 2.26 <sup>Ad</sup>	16.50 ± 1.66 <sup>Ac</sup>	12.36 ± 1.47 <sup>Ab</sup>	8.30 ± 0.66 <sup>a</sup>	5.00 ± 0.27	7.46 ± 0.72
Diabetic/Insulin	IP	23.04 ± 2.31 <sup>Aa</sup>	7.28 ± 0.66	6.48 ± 0.45	6.00 ± 0.35	5.80±0.51	5.46±0.54	6.84±0.48
Extract dose (mg/kg body weight)								
50	Oral	20.28 ± 4.41 <sup>Aa</sup>	12.60 ± 5.56	11.44 ± 3.75	8.74 ± 1.67	8.12 ± 1.23	7.12 ± 1.19	8.22 ± 1.10
	IP	23.44 ± 7.38 <sup>Aa</sup>	21.82 ± 7.50 <sup>Ba</sup>	16.68 ± 4.48 <sup>A</sup>	13.44 ± 3.82 <sup>B</sup>	10.88 ± 4.06 <sup>A</sup>	6.98 ± 1.56	10.74 ± 4.51
100	Oral	26.40 ± 2.49 <sup>Ab</sup>	23.66 ± 2.64 <sup>Ba</sup>	22.90 ± 1.81 <sup>B</sup>	21.50 ± 1.99 <sup>B</sup>	20.06 ± 1.70 <sup>B</sup>	18.54 ± 2.63 <sup>B</sup>	24.58 ± 3.70 <sup>Ba</sup>
	IP	20.94 ± 7.44 <sup>Aa</sup>	18.70 ± 5.42 <sup>B</sup>	17.02 ± 4.21 <sup>A</sup>	12.70 ± 2.90 <sup>A</sup>	8.92 ± 2.31	9.32 ± 0.85 <sup>B</sup>	19.44 ± 9.70 <sup>B</sup>
200	Oral	23.72 ± 5.86 <sup>Ab</sup>	20.22 ± 5.56 <sup>Aa</sup>	16.54 ± 5.57 <sup>A</sup>	15.28 ± 5.68 <sup>A</sup>	12.18 ± 4.94 <sup>A</sup>	9.54 ± 3.93 <sup>A</sup>	14.90 ± 3.55 <sup>A</sup>
	IP	18.56 ± 6.37 <sup>A</sup>	16.10 ± 5.79 <sup>A</sup>	14.08 ± 6.05 <sup>A</sup>	11.32 ± 5.83	8.38 ± 3.40	7.86±1.14 <sup>A</sup>	18.98 ± 9.71 <sup>A</sup>
300	Oral	22.48 ± 5.89 <sup>Aa</sup>	19.32 ± 7.27 <sup>A</sup>	16.50 ± 7.16 <sup>A</sup>	14.86 ± 5.76 <sup>A</sup>	11.62 ± 4.13 <sup>A</sup>	9.40±1.74 <sup>A</sup>	14.42 ± 3.99 <sup>A</sup>
	IP	22.78 ± 3.30 <sup>Ab</sup>	19.66 ± 3.82 <sup>a</sup>	17.10 ± 3.75 <sup>A</sup>	15.12 ± 4.66 <sup>B</sup>	12.84 ± 3.60 <sup>B</sup>	8.30±0.87 <sup>A</sup>	14.96 ± 7.82

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 ≤ 0.05 by ANOVA and post ANOVA; means within respective rows followed by similar lower case letters are not significantly different at p ≤ 0.05 by ANOVA and post ANOVA.

**Table 2:** Effect of oral and intraperitoneal administration of aqueous leaf extracts of *Zanha africana* on blood glucose levels in alloxan induced diabetic mice.



**Figure 1:** The mean percentage change in blood glucose levels of aqueous leaf extracts of *Zanha africana* administered orally in alloxan induced diabetic mice.



**Figure 2:** The mean percentage change in blood glucose levels of aqueous leaf extracts of *Zanha africana* administered intraperitoneally in alloxan induced diabetic mice.

weight decreased blood glucose levels to normal and was effective as insulin while the dose level 200 mg/kg body weight decreased blood glucose levels to normal but was not effective as insulin. However the dose levels 100 mg/kg body weight and 300 mg/kg body weight did not lower blood glucose levels to normal. After this, a gradual increase was recorded up to the twenty fourth hour.

### Effect of oral and intraperitoneal administration of 1 g/kg body weight of aqueous leaf extracts of *Zanha africana* on body and organ weights in mice

Table 3 shows the effect of oral and intraperitoneal administration of aqueous leaf extracts of *Zanha africana* at 1 g/kg body weight to

mice for one month on the weekly changes in body weight and percent organ to body weight. Oral and intraperitoneal administration of 1 g/kg body weight of aqueous leaf extracts of *Zanha africana* to mice for one month significantly decreased the weekly body weight gain relative to that of the normal control mice (Table 3). Oral administration of aqueous leaf extracts of *Zanha africana* at 1 g/kg body weight to mice for one month did not significantly alter the percent organ to body weights of all the studied organs relative to those of the normal control mice (Table 3). In addition, administration of the same intraperitoneal dose of aqueous leaf extracts of *Zanha africana* to mice for one month significantly increased the percent organ to body weight of liver, brain, and kidney but did not significantly alter the percent organ to body weight of the lungs, spleen, heart, and testes relative to those of the normal control mice (Table 3).

### Effect of oral and intraperitoneal administration of 1 g/kg body weight of aqueous leaf extracts of *Zanha africana* on hematological parameters in mice

Results are shown in Table 4. Oral administration of 1 g/kg body weight of aqueous leaf extracts of *Zanha africana* to mice for one month significantly increased the level of MCH and significantly decreased the level of PLT but did not significantly change the levels of RBC, Hb, PCV, MCV, and MCHC relative to those of the normal control mice. In addition, administration of the same intraperitoneal dose of aqueous leaf extracts of *Zanha africana*, 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 1 g/kg body weight of aqueous leaf extracts of *Zanha africana* on white blood cell count in mice

Oral administration of aqueous leaf extracts of *Zanha africana* at 1g/kg body weight to mice for one month did not cause significant change to the differential white blood cell count (Table 5). In addition, Intraperitoneal administration of the same dose of aqueous leaf extracts of *Zanha africana* to mice for one month significantly increased the levels of WBC and Lymphocytes without significantly affecting the levels of Neutrophils, Eosinophils, Monocytes, and Basophils relative to those of normal control mice (Table 5).

### Effects of oral and intraperitoneal administration of 1 g/kg body weight of aqueous leaf extracts of *Zanha africana* on biochemical parameters in mice

Oral administration of 1g/kg body weight of aqueous leaf extracts of *Zanha africana* caused a significant increase in levels of  $\gamma$ -GT, LDH, and CK while significantly decreasing the levels of Urea, ALT, AST, T-BIL, D-BIL and Creatinine relative to that of the normal control mice; however, no significant alteration in the levels of ALP and  $\alpha$ -AMY by the same extract dose compared to that of the respective normal control group (Table 6 and 7). Intraperitoneal administration of the same dose of aqueous leaf extracts of *Zanha africana* significantly increased the levels of  $\gamma$ -GT, T-BIL and D-BIL while decreasing the levels of AST and Creatinine relative to that of the normal control mice; however, no significant alteration on the levels of Urea, ALT, LDH, CK, ALP, and  $\alpha$ -AMY by the same extract dose compared to respective normal control group (Table 6 and 7).

### Quantitative analysis of the phytochemical composition of the aqueous leaf extracts of *Zanha africana*

The results of quantitative analysis of five major groups of phytochemical constituents in the aqueous leaf extracts of *Zanha Africana* are shown in Table 8.

### Mineral elements analysis

Aqueous leaf extracts of *Zanha Africana* contained Sodium (Na), Chlorine (Cl), Potassium (K), Calcium (Ca), Titanium (Ti), Vanadium (V), Chromium (Cr), Manganese (Mn), Iron (Fe), Copper (Cu), Zinc (Zn), Arsenic (As), Cadmium (Cd), Magnesium (Mg), Nickel (Ni) and Lead (Pb). The levels of these measured minerals and trace element were all below the recommended daily allowance (Table 9).

### Discussion

The aim of this study was to investigate the in vivo antidiabetic effect and safety of the aqueous leaf extracts of *Zanha africana* in alloxan induced diabetic mice and normal mice respectively. The alloxan administration resulted in 3 to 4 times increase in blood glucose levels compared to normal control group.

Both oral and intraperitoneal route of administration of the aqueous extract of the studied plant showed hypoglycemic activity

Treatment	Average weekly change in body weight (g) and percent organ to body weight (%)							
	$\Delta$ weight/week	Liver	Brain	Kidney	Lungs	Spleen	Heart	Testes
Control Oral	1.705 $\pm$ 0.522	7.27 $\pm$ 1.12	2.74 $\pm$ 0.27	1.78 $\pm$ 0.24	1.79 $\pm$ 0.21	1.12 $\pm$ 0.32	0.53 $\pm$ 0.09	0.93 $\pm$ 0.16
<i>Zanha africana</i> Oral	0.340 $\pm$ 0.495*	7.83 $\pm$ 1.36	2.65 $\pm$ 0.92	1.87 $\pm$ 0.11	2.28 $\pm$ 0.92	1.05 $\pm$ 0.41	0.62 $\pm$ 0.08	0.92 $\pm$ 0.12
Control IP	2.155 $\pm$ 0.089	5.01 $\pm$ 1.26	1.75 $\pm$ 0.31	1.39 $\pm$ 0.42	1.38 $\pm$ 0.54	0.61 $\pm$ 0.13	0.51 $\pm$ 0.21	0.91 $\pm$ 0.22
<i>Zanha africana</i> IP	0.490 $\pm$ 0.189*	9.18 $\pm$ 1.74*	2.91 $\pm$ 0.37*	2.32 $\pm$ 0.49*	2.19 $\pm$ 0.69	0.93 $\pm$ 0.45	0.71 $\pm$ 0.13	0.94 $\pm$ 0.14

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

**Table 3:** The effects of oral and intraperitoneal administration of aqueous leaf extract of *Zanha africana* at 1 g/kg body weight on body and organ weights in mice.

Treatment	Hematological parameters						
	RBC ( $\times 10^6/\mu\text{L}$ )	Hb (g/dL)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	PLT ( $\times 10^3/\mu\text{L}$ )
Control Oral	7.61 $\pm$ 0.74	9.58 $\pm$ 0.73	32.74 $\pm$ 2.49	43.12 $\pm$ 2.84	12.64 $\pm$ 0.96	29.30 $\pm$ 0.54	607.40 $\pm$ 116.07
<i>Zanha africana</i> Oral	6.69 $\pm$ 1.19	10.08 $\pm$ 1.05	30.96 $\pm$ 5.48	46.26 $\pm$ 2.41	14.40 $\pm$ 1.06*	31.16 $\pm$ 3.21	436.32 $\pm$ 57.54*
Control IP	6.38 $\pm$ 0.67	8.98 $\pm$ 0.80	28.66 $\pm$ 2.64	45.02 $\pm$ 1.44	14.10 $\pm$ 0.51	31.36 $\pm$ 0.66	352.80 $\pm$ 73.32
<i>Zanha africana</i> IP	6.20 $\pm$ 0.66	8.80 $\pm$ 1.10	28.14 $\pm$ 3.25	45.36 $\pm$ 1.93	14.20 $\pm$ 0.72	31.26 $\pm$ 0.61	439.00 $\pm$ 96.11

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 4:** The effects of oral and intraperitoneal administration of 1 g/kg body weight of aqueous leaf extracts of *Zanha africana* on hematological parameters in mice.

Treatment	White blood cell and differential white blood cell count					
	WBC (x10 <sup>3</sup> /μL)	Neutrophils (x10 <sup>3</sup> /μL)	Lymphocytes (x10 <sup>3</sup> /μL)	Eosinophils (x10 <sup>3</sup> /μL)	Monocytes (x10 <sup>3</sup> /μL)	Basophils (x10 <sup>3</sup> /μL)
Control Oral	14.34 ± 3.48	4.46 ± 1.26	7.27 ± 1.68	1.35 ± 0.41	1.15 ± 0.21	0.09 ± 0.08
<i>Zanha Africana</i> Oral	12.82 ± 6.38	3.50 ± 1.70	7.12 ± 3.64	1.17 ± 0.55	0.95 ± 0.43	0.08 ± 0.10
Control IP	6.87 ± 0.71	2.18 ± 0.25	3.51 ± 0.33	0.59 ± 0.14	0.54 ± 0.13	0.04 ± 0.04
<i>Zanha africana</i> IP	9.08 ± 0.94*	2.44 ± 0.32	5.08 ± 0.46*	0.84 ± 0.13	0.71 ± 0.15	0.04 ± 0.05

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 5:** The effects of oral and intraperitoneal administration of 1 g/kg body weight of aqueous leaf extracts of *Zanha africana* on white blood cell count (WBC) in mice.

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 ± 20.6	692.3 ± 51.4	1.8 ± 0.2	1972.9 ± 158.7	953.4 ± 74.7	2940.2 ± 174.7	103.2 ± 9.1
<i>Zanha africana</i> Oral	66.2 ± 7.5*	356.1 ± 42.2*	3.6 ± 0.9*	2182.2 ± 100.4*	1155.6 ± 96.9*	2798.0 ± 311.8	104.7 ± 9.4
Control IP	80.3 ± 7.0	523.2 ± 94.7	2.0 ± 1.0	2137.2 ± 159.4	351.0 ± 59.1	1676.4 ± 230.2	46.6 ± 10.4
<i>Zanha africana</i> IP	98.8 ± 21.5	383.2 ± 48.4*	4.0 ± 1.2*	2087.2 ± 265.8	336.0 ± 92.4	1650.0 ± 209.3	52.6 ± 20.0

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 6:** The effects of oral and intraperitoneal administration of 1 g/kg body weight of aqueous leaf extracts of *Zanha africana* on organ functions in mice.

Treatment	Metabolite Levels			
	UREA (mmole/L)	CREAT (μmole/L)	T-BIL (μmole/L)	D-BIL (μmole/L)
Control Oral	9.6 ± 0.7	75.0 ± 8.5	0.8 ± 0.1	0.4 ± 0.1
<i>Zanha Africana</i> Oral	7.4 ± 0.6*	54.4 ± 3.2*	0.5 ± 0.1*	0.3 ± 0.1*
Control IP	7.2 ± 0.8	43.2 ± 6.1	4.5 ± 1.2	2.6 ± 0.7
<i>Zanha africana</i> IP	8.2 ± 0.8	28.6 ± 3.5*	8.7 ± 0.9*	5.3 ± 0.8*

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 7:** The effects of oral and intraperitoneal administration of 1 g/kg body weight of aqueous leaf extracts of *Zanha africana* on the levels of selected metabolites in mice.

at the four tested dose levels (50 mg/kg body weight 100 mg/kg body weight, 200 mg/kg body weight, and 300 mg/kg body weight). These finding agrees with the results obtained by Mukundi et al. [28] who undertaken a similar study and reported that the aqueous leaf extracts of *Acacia nilotica* showed hypoglycemic activity in alloxan induced diabetes mice.

This hypoglycemic activity could be due to the presence of flavonoids, alkaloids, saponins, tannins and total phenols in the studied plant extract (Table 7). Flavonoids are shown to stimulate peripheral glucose uptake and regulate the activity and/or expression of the rate-limiting enzymes involved in carbohydrate metabolism pathway by acting as insulin secretagogues or insulin mimetics [29] Flavonoids isolated from *Pterocarpus marsupium* has been shown to in vitro cause pancreatic β-cell regeneration and found to enhance insulin release and conversion of proinsulin to insulin [30].

Presence of saponins in this extract could also be responsible for the hypoglycemic activity. For instant total saponins from the seeds of *Entada phaseoloides* showed significant decrease in fasting blood glucose levels in type 2 diabetic rats [31]. In additions saponins were found to reduce serum glucose levels in elderly diabetic patients [32].

It was reported that condensed tannins obtained from some Kenyan foods inhibited of α-amylase and α-glucosidase enzymes [33] in addition, Commercially available tannic acids were shown to induce phosphorylation of the insulin receptor (IR) and cause translocation of glucose transporter 4 (GLUT 4) [34].

Alkaloids are also reported to be antidiabetic. For example alkaloids obtained from leaves of *Acanthus montanus* administered intraperitoneally at doses of 100, 200 and 400mg/kg body weight

demonstrated hypoglycemic activity in alloxan-induced diabetic rats [35]. Four indole Alkaloids isolated from the leaves of *Catharanthus roseus* increased glucose uptake in pancreatic and muscle cells. Moreover these alkaloids were found to inhibit protein tyrosine phosphatase PTP-1B which is a down regulator in the insulin signaling pathway [36].

In addition to phytochemical components, the hypoglycemic activity of the studied plant extract could result from its mineral/trace elements composition. Zinc is required in all aspects of insulin metabolism, synthesis, secretion and utilization. There is a high zinc excretion rate in diabetic patients and zinc supplementation was shown to improve insulin levels in both type 1 and type 2 diabetes [37].

Magnesium plays important role in glucose transport across cell membranes and is found as a cofactor in various enzymes involved in glucose oxidation pathways [38]. It was demonstrated that 4 weeks dietary Mg supplementation improved insulin secretory capacity [39].

Studies on guinea pigs showed that manganese deficiency caused impaired glucose tolerance and this was corrected by manganese supplementation [40]. In vitro and in vivo studies demonstrated that selenium exhibits insulin like activities, such as glucose uptake stimulation and regulation of metabolic pathways like glycolysis, pentose phosphate pathway, fatty acid synthesis and gluconeogenesis [41].

Experimental studies demonstrated that hypokalemia resulting from use of potassium wasting diuretics caused decrease in the pancreatic capacity to secrete insulin, diminished β-cell sensitivity to insulin and hence caused impaired glucose tolerance [42]. However potassium supplementation was found to improve insulin sensitivity, responsiveness and secretion [43,44].

Extract	Phytochemical content				
	Tannins	Total Phenols	Flavonoids	Saponins	Alkaloids
<i>Zanha africana</i>	0.7671 ± 0.004	1.8503 ± 0.077	1.5830 ± 0.342	52.333 ± 3.960	56.520 ± 7.806

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 leaf extracts of *Zanha africana*.

Element	<i>Zanha africana</i> (µg/g)	Amount given to each mouse (µg)	RDA for mice (µg/day)*
Na*	1893.3 ± 128.2	47.3325	5 × 10 <sup>5</sup> (178.6)
Mg*	205.9 ± 41.7	5.1475	4.2 × 10 <sup>5</sup> (150)
Cl*	143.2 ± 1.9	3.58	7.5 × 10 <sup>5</sup> (267.9)
K*	1474.6 ± 13.7	36.865	3.5 × 10 <sup>6</sup> (1250)
Ca*	50.8 ± 0.6	1.27	1.0 × 10 <sup>6</sup> (357.1)
Ti	0.41 ± 0.05	0.01025	
V	0.16 ± 0.03	0.004	<1.8 × 10 <sup>3</sup> (<0.64)
Cr*	0.012 ± 0.006	0.0003	3.5 × 10(12.5)
Mn*	2.41 ± 0.05	0.06025	2.3 × 10 <sup>3</sup> (0.82)
Fe*	16.29 ± 0.19	0.40725	8.0 × 10 <sup>3</sup> (2.9)
Ni	0.57 ± 0.02	0.01425	<1 × 10 <sup>3</sup> (<0.36)
Cu*	0.23 ± 0.01	0.00575	1.5 × 10 <sup>3</sup> (0.54)
Zn*	1.36 ± 0.03	0.034	1.1 × 10 <sup>4</sup> (3.9)
As	0.05 ± 0.01	0.00125	
Se	<0.030	<0.00075	3.5 × 10(0.0125)
Pb	0.08 ± 0.01	0.002	
Cd	7 ± 0.9	0.175	

Results on the concentration of each mineral are expressed as µg/g of dry powder of the aqueous leaf extracts of *Zanha africana* 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 recommended daily allowance 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 Strain and Cashman (2009) [60].

**Table 9:** Mineral levels and amount given to each mouse from the aqueous leaf extracts of *Zanha africana*

The trivalent Cr is a part in biologically active substance called glucose tolerance factor (GTF), that regulates glucose biotransformation and increases the number of insulin receptors, enhances receptor binding, and potentiates insulin action [37,45]. Experimental chromium deficiency was found to lead to impaired glucose tolerance, which is improved by chromium supplementations [38,45].

Calcium improves insulin sensitivity in some type 2 diabetic patients [46]. Vanadium acts as phosphate analog and exerts effects on several steps in the insulin signaling pathways [45]. Animal model studies showed that vanadium enhanced insulin sensitivity and increased glucose uptake [47,48].

The oral and intraperitoneal administration of the aqueous leaf extracts of *Zanha africana* caused decrease in growth rate. This decrease in growth rate could be due to the presence of alkaloids, saponins, flavonoids, and tannins. For instance, flavonoids decrease body weight through decreasing glucose absorption. This leads to an increase in fat oxidation. Catechins (flavanoids) are reported to reduce body weight possible by two mechanisms: Inhibition of small-intestine micelle formation and inhibition of α-glucosidase activity which would lead to a decrease in carbohydrate absorption [49].

Alkaloids which are present in high amounts in the aqueous extracts of *Zanha africana* are found to cause weight loss. Alkaloids like Synephrines and ρ-octopamine cause a decrease in body weight by

increasing resting energy expenditure, energy used in physical activity and thermal effect of feeding, by 70%, 20% and 10% respectively [49]. Nicotine an alkaloid mainly found in tobacco plant has been reported to act on the central nervous system and modulate several pathways that regulate the aspects of food intake leading to reduced appetite. Cathinone (monoamine alkaloid) delays gastric emptying and hence reduces appetite by acting on the hypothalamus [49].

For the extract administered through the oral route, tannins are reported to reduce feed intake by decreasing palatability and by reducing feed digestion. Palatability is reduced because astringency effect of tannins. Astringency is the sensation resulting from formation of complexes between tannins and salivary glycoproteins. Reduced palatability depresses feed intake. Reduction in digestibility negatively affects intake by causing filling effect due to presence of undigested food [50].

Proanthocyanidins which are condensed tannins cause damage to the mucosa of the gastrointestinal tract resulting in decreased absorption of nutrients. They are also found to increase excretion of proteins and essential amino acids [51]. Mineral element overdose may also cause toxicity but this was not the case with the measured minerals since their levels were below the recommended daily allowance.

The increased percent organ to body weight of liver, brain and kidneys of mice intraperitoneally administered with the aqueous leaf extracts of *Zanha africana* at 1 g/kg body weight daily for one month could not be explained in this study. It is possible that the extract promoted higher metabolic activity in these organs.

The investigated hematological parameters in this study are important in the assessment of the potential toxic effect of the plant extract on the bone marrow activity and hemolysis [52]. The main reason for measuring RBC is to check anemia and to evaluate erythropoiesis. Hemoglobin level indicates oxygen carrying capacity of the body, while packed cell volume helps to determine the degree of anemia or polycythaemia. The mean cell hemoglobin level is an important index for folic acid and or vitamin B<sup>12</sup> need. Platelets are important for blood clotting, they initiate repair of blood vessels walls and act as an acute phase reactant to infection or inflammation [53].

Studies have shown that use of medicines or herbal drugs can alter the normal range of hematological parameters [54]. In the present study the oral administration of aqueous leaf extracts of *Zanha africana* caused an increase in MCH levels and decreased platelet count. This may be due to the toxic constituents in this plant extracting including; total phenols, alkaloids, saponins, flavonoids, and tannins present in this plant extracts.

Saponins hemolyse and cause cell death in many tissues [55,56]. Alkaloids have been shown to cause liver megalocytosis, proliferation of biliary tract epithelium, liver cirrhosis and nodular hyperplasia [57]. This toxicity may not have been due to the presence of trace elements/minerals since the amounts administered into each mouse daily at a dose of 1g/kg body weight were below the recommended daily allowance.

The significant increase in white blood cells observed on intraperitoneal administration of plant extracts of *Z. africana* indicates a more accelerated production of these cells and a boosted immunity to mice treated by this extract [58-60]. This could be due to tissue damage caused by some constituents of this plant extract.

This argument is in line with the observed enlargement of the liver, brain and kidney and the altered levels of alanine transaminase, aspartate aminotransferase,  $\gamma$ -glutamyl transpeptidase, lactate dehydrogenase, creatine kinase, urea, creatinine, total bilirubin and direct bilirubin in mice administered with 1g of *Z. africana* extracts/kg body weight.

The observed significant increase in lymphocytes (main effectors cells of the immune system) on intraperitoneal administration of aqueous extracts of *Z. africana* at 1 g/kg body weight in mice for 28 days indicates a possible stimulatory effect by this extract on lymphocyte production.

## Conclusion

The aqueous leaf extracts of *Zanha africana* had antidiabetic activity. The aqueous extract of the studied plant at high dose of 1 g/kg body weights which is far from the therapeutic dose tends to cause toxicological effects. This was well demonstrated in the body and organ weight changes, hematological, and biochemical parameters. In the toxicological studies the oral administration of the high dose (1 g/kg body weight) was found to have less toxic effects than the intraperitoneal administration of the same dose. This explains why the oral route is the most preferred route by the traditional health practitioners. The antidiabetic and toxic action of the studied plant may have resulted from its phytochemical and mineral constituents.

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