

In-Vivo Anti-hyperglycemic Activity and Safety of The Aqueous Stem Bark Extracts of *Aloe secundiflora*

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Abstract

Aloe secundiflora 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 stem bark 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 of the extract 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 significant and consistent hypoglycemic activity at dose levels of 50 mg/kg body weight through oral route and 300 mg/kg body weight through intraperitoneal route. Oral administration of 1 g/kg body weight of the extract decreased levels of platelets, alanine transaminase, aspartate aminotransferase, alkaline phosphatase, creatinine and direct bilirubin while elevated the level of creatine kinase. Reduction in the growth rate and increase in percent of organ to body weight of brain together with elevated levels of mean corpuscular hemoglobin concentration, γ -glutamyl transpeptidase, α -amylase, alkaline phosphatase, total bilirubin and direct bilirubin were recorded in mice intraperitoneally administered with 1 g/kg body weight of the extract. The extracts contained tannins, phenols, flavonoids, saponins, and alkaloids. Sodium, Chlorine, Potassium, Calcium, Titanium, Vanadium, Chromium, Manganese, Iron, Copper, Zinc, Arsenic, Cadmium, and Magnesium 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: *Aloe secundiflora*; Diabetes mellitus; Biochemical parameters; Hematological parameters; Hypoglycemic effect; Phytochemicals

Introduction

Diabetes mellitus is one of the most common metabolic disorders which is characterized by hyperglycemia and associated with serious micro vascular and macro vascular complications like retinopathy, neuropathy, nephropathy, cardiovascular disorders and peripheral vascular complications with associated risk for foot ulcers and amputation [1]. According to a recent estimation by the World Health Organization (WHO) diabetes mellitus has a global prevalence of 9% among adults. The disease has caused over 1.5 million deaths in 2012 and WHO projects that diabetes will be the 7th leading cause of death in 2030 [2].

In Kenya a recent study has shown 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 [3]. The number of people with diabetes is increasing due to population growth, aging, urbanization and increasing prevalence of obesity and physical inactivity [4].

Although diabetes is sometimes considered a condition of developed nations, the loss of life from premature death among persons with diabetes is greatest in developing countries. In developing countries it is people in the middle, productive years of their lives that are particularly affected by diabetes. In these countries three-quarters of all people with diabetes are under 65 years old and 25% of all adults with diabetes are younger than 44 years. In developed countries, more than half of all people with diabetes are older than 65 years, and only 8% of adults with diabetes are younger than 44 years [5].

Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides and glinides. Many of them have a number of serious adverse effects, therefore, the search for more effective and safer hypoglycemic agents is one of the important areas of investigation [6]. A notable number of plants have been described for the treatment of diabetes throughout the world and the hypoglycemic action of some these plants has been confirmed in animal models and non-insulin dependent diabetic patients [7].

Aloe species have been valued since prehistoric times as medicine for the treatment of burns, wound infections and other skin problems. A recent review of some *aloe* species showed that they have antibacterial, antifungal, antiviral, anticancer, anti-diabetes and immunomodulatory properties [8].

Aloe secundiflora is traditionally used to treat painful diaphragm, malaria, edema, nose bleeding, diarrhea, and typhoid fever [9]. It was demonstrated that methanol extract of *Aloe secundiflora* exhibits

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strong antibacterial activities against a number of microorganisms including four strains of mycobacteria (*M. tuberculosis*, *M. kansasii*, *M. fortuitum* and *M. smegmatis*), *Salmonella typhi*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumonia* [10].

It was observed that crude extracts of *Aloe secundiflora* mature leaves showed antimicrobial effect on *Candida albicans* a fungus that is the second most cause of vaginal irritation or vaginitis and can also occur in male genital organ [11]. *Aloe secundiflora* has also ethno-veterinary properties. It was reported that the crude extract of the plant delayed the occurrence of the clinical signs and reduced severity of the disease in experimentally infected chickens with *Salmonella gallinarum* (bacteria that causes Fowl typhoid) [8].

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 November 2012 to August 2014. Kenyatta University is 23 km from Nairobi off Thika Road.

Collection of the plant materials

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 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 plant

The collected plant materials were chopped into small pieces, dried under shade at room temperature for 1 month, and then ground when completely dry using an electric mill. The powdered plant materials were kept at room temperature away from direct sunlight in closed dry plastic bags.

Preparation of the aqueous extracts

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 h. 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 h/12 h 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 (Steinhein, Switzerland) [12].

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 h [13] 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 0.1 ml physiological saline; Group IIIa consisted of alloxan induced diabetic mice intraperitoneally administered with insulin at (1 I U/kg body weight) in 0.1 ml physiological saline; Group IIIb consisted of alloxan induced diabetic mice orally administered with glibenclamide at (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 the extract at (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 the extract at (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 the extract at (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 the extract at (300 mg/kg body weight) in 0.1 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 blood 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 h. 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, respectively, 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 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 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 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 h 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 [14]. 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 [14]. 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), γ -glutamyl transpeptidase (γ -GT), 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 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 phytochemical screening

The extract was screened for the presence of five major classes of phytochemicals using the recommended procedures. Alkaloids [15] saponins [16] Flavonoids [17] phenolis [16] and Tanins [17].

Quantitative phytochemical screening

The phytochemicals present were quantified using standard procedures. Alkaloids [18] Saponins [19] Flavonoids [20], Phenols [21] and Tannins [22].

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 [23]. Atomic absorption spectrometry (AAS) was used for the analysis of Magnesium, Chromium and Cadmium [24]. 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 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 stem bark extracts of *Aloe secundiflora* on blood glucose levels in alloxan induced diabetic mice.

The dry powder of *Aloe secundiflora* yielded 7.55% (w/w) aqueous stem bark extract. Table 1 and Figures 1 and 2 show the pattern of blood glucose reduction by four aqueous stem bark extract doses (50, 100, 200 and 300 mg/kg body weight) of *Aloe secundiflora*. In the 1st h, oral administration of the four aqueous stem bark extract doses (50, 100, 200 and 300 mg/kg body weight) caused a percent reduction in the blood glucose levels of 16.48%, 6.71%, 15.19% and 7.69%, respectively, compared to the reference drug glibenclamide which lowered blood glucose levels by 8.28% within the same hour. As Table 1 shows, at this hour, the aqueous stem bark extract dose levels of 100, 200, and 300 mg/kg body weight insignificantly lowered blood glucose levels. However the aqueous extract dose level of 50 mg/kg body weight significantly lowered the blood glucose levels in the 1st h. In the 6th h the percent blood glucose reductions by the four aqueous extract doses were 63.83%, 10.89%, 36.60% and 9.11%, respectively, compared to glibenclamide which lowered blood glucose levels by 78.56% within the same hour. At this hour only the dose level 50 mg/kg body weight significantly lowered blood glucose levels while the other doses insignificantly lowered the blood glucose levels at this hour. After this, a gradual increase was recorded up to the twenty fourth hour.

As Table 1 shows after intraperitoneal administration of stem bark extract of *Aloe secundiflora* only the dose level 300 mg/kg body weight had shown significant and consistence reduction in the blood glucose levels. The percent blood glucose reductions of this aqueous stem bark extract dose were 60.96% in the 1st h and 68.57% in the 6th h After this, a gradual increase was recorded in the 24th h. The aqueous stem bark extract dose level of 50 mg/kg body weight significantly lowered blood glucose levels by 15.50% in the 1st h. After this, a gradual increase was recorded from 2nd to 6th h. In the 24th the dose again significantly lowered blood glucose levels by 60%. The aqueous stem bark extract dose level at 100 mg/kg body weight did not significantly lower the blood glucose levels from 1st to 6th h. However the dose insignificantly lowered blood glucose levels by 27.26% in the 24th h. The aqueous stem

bark extract dose level of 200 mg/kg body weight had only shown insignificant reduction in blood glucose levels by 12.59% and 12.18% in the 6th and 24th h respectively.

Effect of oral and intraperitoneal administration of 1 g/kg body weight of aqueous stem bark extracts of *Aloe secundiflora* on body and organ weights in mice.

Table 2 show the effect of oral and intraperitoneal administration of the aqueous stem bark extracts of *Aloe secundiflora* at 1 g/kg body weight to mice for one month on the weekly change in body weight and percent organ to body weight. Oral administration 1 g/kg body weight of aqueous stem bark extracts of *Aloe secundiflora* to mice for one month did not significantly alter the weekly body weight changes and percent organ to body weights of all the studied organs relative to those of the normal control mice (Table 2). In addition administration of the same intraperitoneal dose of aqueous stem bark extracts of *Aloe secundiflora* to mice for one month significantly decreased the weekly body weight changes and increased the percent organ to body weight of brain while not significantly altering the percent organ to body weight of the liver, kidney, lungs, spleen, heart, and testes relative to those of the normal control mice (Table 2).

Effect of oral and intraperitoneal administration of 1 g/kg

Treatment	Route	Glucose levels at varying times in mmole/L						
		0hr	1hr	2hr	3hr	4hr	6hr	24hr
Normal control	Oral	5.22 ± 0.43	5.26 ± 0.24	5.32 ± 0.28	5.20 ± 0.32	5.28 ± 0.22	5.08 ± 0.29	5.22 ± 0.15
	IP	5.16 ± 0.11	5.16 ± 0.11	5.14 ± 0.11	5.14 ± 0.19	5.14 ± 0.11	5.12 ± 0.13	5.12 ± 0.18
Diabetic control	Oral	27.02 ± 1.28 ^A	27.44 ± 0.92 ^B	27.88 ± 0.86 ^B	28.58 ± 0.59 ^C	28.98 ± 0.51 ^{Ca}	29.62 ± 0.61 ^{Bb}	30.52 ± 0.87 ^{Cc}
	IP	22.64 ± 2.63 ^A	23.76 ± 2.70 ^A	24.66 ± 2.87 ^A	25.70 ± 2.36 ^A	26.86 ± 1.77 ^A	28.10 ± 1.40 ^{Ba}	29.48 ± 0.99 ^{Bb}
Diabetic/Gliben	Oral	25.84 ± 2.37 ^{Ac}	23.70 ± 2.56 ^{Ac}	18.44 ± 2.98 ^{Ab}	11.12 ± 2.02 ^a	6.84 ± 1.26	5.54 ± 0.59	7.02 ± 0.37
Diabetic/Insulin	IP	22.64 ± 1.60 ^{Ab}	6.94 ± 0.42 ^a	6.58 ± 0.45	6.20 ± 0.35	5.70 ± 0.51	5.40 ± 0.57	6.70 ± 0.48
Extract dose (mg/kg body weight)								
50	Oral	18.08 ± 5.98 ^{Ab}	15.10 ± 6.67	12.96 ± 7.03	11.34 ± 5.31	10.44 ± 4.73	6.54 ± 1.56	9.16 ± 4.31
	IP	25.16 ± 2.63 ^{Ab}	21.26 ± 4.86 ^A	22.56 ± 5.43 ^{Ab}	25.38 ± 5.49 ^{Ab}	23.26 ± 5.41 ^{Ab}	25.30 ± 7.24 ^{Ab}	10.06 ± 7.41
100	Oral	25.34 ± 7.54 ^A	23.64 ± 7.82 ^A	21.86 ± 7.32 ^A	22.30 ± 7.01 ^B	23.14 ± 6.76 ^B	22.58 ± 6.34 ^A	24.60 ± 6.26 ^B
	IP	21.86 ± 5.73 ^A	24.18 ± 4.55 ^A	25.44 ± 4.39 ^A	26.70 ± 6.44 ^{Ab}	26.10 ± 5.83 ^A	26.58 ± 6.27 ^B	15.90 ± 3.62 ^A
200	Oral	21.86 ± 6.03 ^A	18.54 ± 5.90 ^A	18.40 ± 6.56 ^A	15.60 ± 6.90 ^A	14.32 ± 6.78	13.86 ± 6.79	16.86 ± 6.47 ^A
	IP	19.70 ± 3.57 ^A	21.50 ± 4.03 ^A	20.56 ± 4.97 ^A	21.38 ± 6.39 ^A	20.38 ± 5.45 ^A	17.22 ± 6.48 ^A	17.30 ± 5.53 ^A
300	Oral	19.76 ± 8.60 ^A	18.24 ± 9.50 ^A	18.12 ± 8.49 ^A	16.98 ± 7.28 ^A	18.24 ± 7.23 ^A	17.96 ± 7.44 ^A	19.02 ± 8.11 ^B
	IP	19.98 ± 6.47 ^{Ab}	7.80 ± 1.38	8.36 ± 1.78	7.44 ± 1.55	7.22 ± 1.79	6.28 ± 0.93	7.62 ± 2.02

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

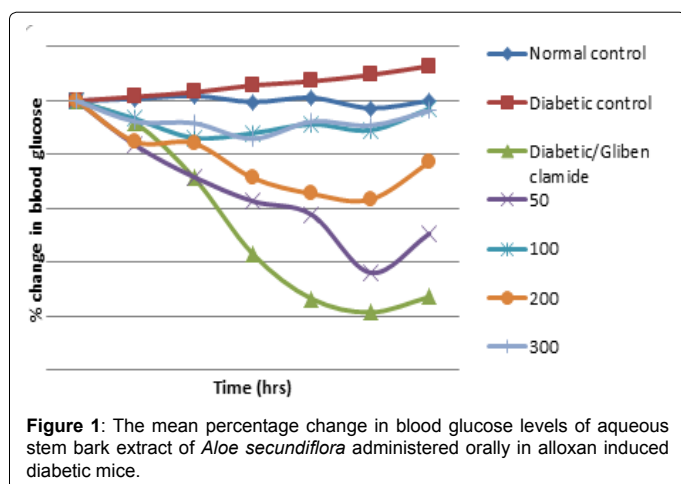


Figure 1: The mean percentage change in blood glucose levels of aqueous stem bark extract of *Aloe secundiflora* administered orally in alloxan induced diabetic mice.

body weight of aqueous stem bark extracts of *Aloe secundiflora* on hematological parameters in mice.

Table 3 shows the effect of oral and intraperitoneal administration of the aqueous stem bark extracts of the studied plant for one month on some hematological parameters in mice. Oral administration of 1 g/kg body weight of *Aloe secundiflora* aqueous stem bark extracts to mice for one month significantly decreased the level of PLT, but did not significantly change the levels of RBC, Hb, PCV, MCV, MCH, and MCHC relative to those of the normal control mice (Table 3). In addition, administration of the same intraperitoneal dose of aqueous stem bark extracts of *Aloe secundiflora* to mice for one month significantly increased the level of MCHC but did not significantly change the levels of RBC, HGB, PCV, MCV, MCH, and PLT relative to those of the normal control mice (Table 3).

Effect of oral and intraperitoneal administration of 1 g/kg body weight of aqueous stem bark extracts of *Aloe secundiflora* on white blood cell count in mice.

Table 4 shows the effect of oral and intraperitoneal administration of the aqueous stem bark extracts of the studied plant at 1 g/kg body weight to mice for one month on differential white blood cell count.

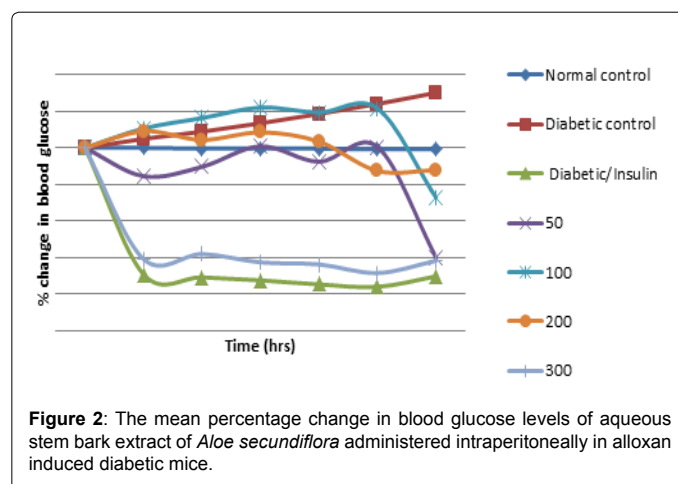


Figure 2: The mean percentage change in blood glucose levels of aqueous stem bark extract of *Aloe secundiflora* administered intraperitoneally in alloxan induced diabetic mice.

Treatment	Weekly body weight change (g) and Percent organ to body weight (%)							
	Δweight/week	Liver	Brain	Kidney	Lungs	Spleen	Heart	Testes
Control Oral	1.705 ± 0.522	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>Aloe secundiflora</i> Oral	1.235 ± 0.775	7.73 ± 1.25	2.85 ± 0.52	1.78 ± 0.23	2.03 ± 0.48	1.35 ± 0.28	0.58 ± 0.04	0.82 ± 0.18
Control IP	2.155 ± 0.089	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>Aloe secundiflora</i> IP	1.120 ± 0.048*	6.47 ± 0.71	2.50 ± 0.49*	1.59 ± 0.16	1.24 ± 0.08	0.41 ± 0.25	0.52 ± 0.13	0.89 ± 0.10

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 aqueous stem bark extract of *Aloe secundiflora* at 1 g/kg body weight on body and organ weights in mice.

Treatment	Hematological parameters						
	RBC (x10 ⁹ /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>Aloe secundiflora</i> Oral	6.07 ± 2.89	8.08 ± 3.83	27.34 ± 12.37	45.72 ± 2.29	13.26 ± 0.81	29.04 ± 2.03	418.98 ± 77.67*
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>Aloe secundiflora</i> IP	5.66 ± 1.03	8.04 ± 1.27	25.06 ± 3.45	44.96 ± 2.88	14.62 ± 0.67	32.60 ± 0.95*	354.80 ± 58.28

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 3: The effects of oral and intraperitoneal administration of 1 g/kg body weight of aqueous stem bark extracts of *Aloe secundiflora* on hematological parameters in mice.

Treatment	White blood cell and differential white blood cell count					
	WBC (x10 ³ /μL)	Neutrophils (x10 ³ /μL)	Lymphocytes (x10 ³ /μL)	Eosinophils (x10 ³ /μL)	Monocytes (x10 ³ /μL)	Basophils (x10 ³ /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>Aloe secundiflora</i> Oral	9.10 ± 8.24	2.70 ± 2.39	4.70 ± 4.28	0.87 ± 0.82	0.73 ± 0.66	0.10 ± 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>Aloe secundiflora</i> IP	7.08 ± 1.47	2.13 ± 0.45	3.69 ± 0.76	0.65 ± 0.13		0.05 ± 0.04

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 *Aloe secundiflora* on white blood cell count (WBC) in mice.

Oral and intraperitoneal administration of aqueous stem bark extracts of *Aloe secundiflora* at 1 g/kg body weight to mice for one month did not cause significant change 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 *Aloe secundiflora* on biochemical parameters in mice.

Oral administration of 1 g/kg body weight of aqueous stem bark extracts of *Aloe secundiflora* to mice for one month caused a significant increase in the level CK and a significant decline in the levels of ALT, AST, ALP, D-BIL and creatinine relative to that of the normal control mice; however, the levels of Urea, γ-GT, LDH, α-AMY, and T-BIL were similar to those of the normal control mice (Tables 5 and 6). Intraperitoneal administration of the same dose of aqueous stem bark extracts of *Aloe secundiflora* to mice for one month increased the levels of γ-GT, AMY, ALP, T-BIL and D-BIL but did not significantly alter the levels of Urea, ALT, AST, LDH, CK, and creatinine relative to that of the normal control mice (Tables 5 and 6).

Quantitative analysis of the phytochemical composition of the aqueous stem bark extracts of *Aloe secundiflora*

The phytochemical analysis indicated the presence of alkaloids, tannins, flavonoids, saponins and total phenols in the aqueous stem bark extract of *Aloe secundiflora*. The amount of each phytochemical per gram of the extract is shown in Table 7.

Mineral elements analysis

Table 8 shows the mineral composition of the studied plant. 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) and Magnesium (Mg) were present in the aqueous stem bark extract of *Aloe secundiflora*. The levels of these measured minerals and trace element were all below the recommended daily allowance.

Discussion

The present study investigated the antidiabetic effect of the aqueous stem bark extract of *Aloe secundiflora* collected from Machakos county Kenya in alloxan induced diabetic mice. The safety of the extract was screened in normal mice. Alloxan caused 3-4 fold increase in glucose levels compared to the normal control mice. The extract showed significant and consistent hypoglycemic activity only at the dose level 50 mg/kg body weight through the oral route and at the dose level of 300 mg/kg body weight through the intraperitoneal route.

These hypoglycemic activities are in line with the finding of similar studies on the blood glucose lowering effect of medicinal plants like *Kleinia squarrosa* and *Acacia nilotica* collected from the same source as the studied plant [25,26]. In addition [27] reported the antidiabetic effect of five Kenyan medicinal plants collected from Eastern Province, Kenya in alloxan induced diabetic mice.

The hypoglycemic effect of the studied plant extract could be attributed to the presence of the phytochemicals like flavonoids, alkaloids, tannins, saponins. Several polyphenols, especially flavonoids, phenolic acids and tannins, are reported to inhibit of α-glucosidase and α-amylase, the key enzymes responsible for digestion of dietary carbohydrates to glucose [28]. Daily administration of a flavonoids-rich fraction isolated from guava leaves at a dose of 7.2-14.4 g

Treatment	Enzyme Activities						
	ALT (U/L)	AST (U/L)	γ-GT (U/L)	LDH (U/L)	CK (U/L)	α-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>Aloe secundiflora</i> Oral	66.2 ± 10.0*	406.4 ± 36.3*	2.0 ± 0.4	1935.4 ± 221.5	2603.6 ± 111.2*	2916.8 ± 276.4	69.0 ± 9.2*
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>Aloe secundiflora</i> IP	93.6 ± 13.7	513.6 ± 131.1	3.6 ± 0.6*	2127.4 ± 208.4	396.8 ± 151.4	2397.4 ± 324.3*	62.0 ± 9.5*

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 stem bark extracts of *Aloe secundiflora* 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>Aloe secundiflora</i> Oral	8.8 ± 1.0	44.8 ± 2.6*	0.7 ± 0.1	0.2 ± 0.1*
Control IP	7.2 ± 0.8	43.2 ± 6.1	4.5 ± 1.2	2.6 ± 0.7
<i>Aloe secundiflora</i> IP	7.9 ± 1.1	47.2 ± 10.8	8.9 ± 1.6*	5.3 ± 0.6*

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 stem bark extracts of *Aloe secundiflora* on the levels of selected metabolites in mice.

Phytochemical	Amount (mg/g)
Tannins	7.8458 ± 0.243
Total Phenols	0.2921 ± 0.083
Flavonoids	5.9588 ± 0.323
Saponins	73.867 ± 5.280
Alkaloids	47.120 ± 8.259

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 7: Quantitative analysis of the phytochemical composition of aqueous stem bark extracts of *Aloe secundiflora*.

lowered blood glucose in humans [29]. In addition Supplementation with hesperidin and naringin, two main citrus bioflavonoids, was accompanied with increased hepatic glucokinase activity and glycogen content, attenuated hepatic gluconeogenesis by decreasing the activity of glucose-6-phosphatase and phosphoenolpyruvate carboxykinase (PEPCK), and subsequent improvement of glycemic control in diabetic rats [28].

Alkaloids are also shown to have antidiabetic effect. 1-Ephedrine (an alkaloid) is shown to promote the regeneration of pancreas islets following atrophy, restoring the secretion of insulin, and thus correct hyperglycemia [30]. It was found that the neem seed kernel powder contained hypoglycemic alkaloids that lowered blood sugar levels in alloxan-induced diabetic rabbits [31]. The extract also contained tannins which are known to have hypoglycemic activity. The tannin epigallo-catechin-3-gallate exhibits antidiabetic activity as demonstrated by [32].

Saponins isolated from the leaves of *Acanthopanax senticosus* intraperitoneally administered at doses of (100 and 200 mg/kg) have been shown to decrease hyperglycemia induced by injection of adrenaline, glucose and alloxan, without affecting the blood glucose levels of untreated mice [33]. Ginsenoside Re (a saponin), exhibited antidiabetic activity by reducing insulin resistance through activation of PPAR-γ pathway, by directly increasing the expressions of PPAR-γ₂ and its responsive genes in 3T3-L1 adipocytes [34].

Apart from these phytochemicals the hypoglycemic activity of the

Element	<i>Aloe secundiflora</i> (μg/g)	Amount given to each mouse (μg)	RDA for mice (μg/day)*
Na	1766.0 ± 242.8	44.15	5 × 10 ⁵ (178.6)
Mg*	221.8 ± 74.2	5.545	4.2 × 10 ⁵ (150)
Cl	95.58 ± 2.81	2.3895	7.5 × 10 ⁵ (267.9)
K	1107.1 ± 19.8	27.6775	3.5 × 10 ⁶ (1250)
Ca	190.42 ± 3.84	4.7605	1.0 × 10 ⁶ (357.1)
Ti	1.1 ± 0.1	0.0275	
V	0.3 ± 0.1	0.0075	< 1.8 × 10 ³ < (0.64)
Cr*	0.009 ± 0.003	0.000225	3.5 × 10 (12.5)
Mn	6.5 ± 0.2	0.1625	2.3 × 10 ³ (0.82)
Fe	14.00 ± 0.33	0.35	8.0 × 10 ³ (2.9)
Cu	0.25 ± 0.03	0.00625	1.5 × 10 ³ (0.54)
Zn	1.49 ± 0.05	0.03725	1.1 × 10 ⁴ (3.9)
As	0.04 ± 0.01	0.001	
Se	< 0.030	<0.00075	3.5 × 10 (0.0125)
Hg	< 0.050	<0.00125	
Cd*	1 ± 0.3	0.025	

Results on the concentration of each mineral are expressed as μg/g of dry powder of the aqueous stem bark extracts of *Aloe secundiflora* 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 by [54].

Table 8: Mineral levels and amount given to each mouse from the aqueous stem bark extracts of *Aloe secundiflora*.

extract could be due to its mineral constituents. Zinc is important for the proper functioning of insulin in the body [35]. Zinc deficient is associated with diabetes due to the increased urinary zinc excretion in diabetic patients and Zinc supplements are shown to lower blood sugar levels in people with IDDM [36].

Manganese is a constituent of some enzymes and an activator to others [37]. These enzymes include Pyruvate carboxylase (a manganese-containing enzyme) and phosphoenolpyruvate carboxykinase (a manganese-activated enzyme) which play critical roles in gluconeogenesis; the production of glucose from non-carbohydrate precursors [38]. Moreover manganese metal is used in making and activation of superoxide dismutase an antioxidant enzyme that help to protect the cell membranes and tissue from degeneration and disruption [39].

Chromium is a trace element required for the maintenance of normal glucose metabolism [40]. Chromium promotes insulin dependent glucose uptake into the cells for energy. Chromium supplements are shown to reduce blood glucose levels in individuals with type 2 diabetes and reduce the need for insulin in those with type 1 diabetes [41].

Vanadium mimics the action of insulin and in a number of human studies; vanadyl sulfate (a form of vanadium) increases insulin sensitivity in those with type 2 diabetes. Vanadium lowers blood glucose to normal levels (reducing the need for insulin) in diabetics [36,42].

Hypomagnesemia is common in patients with diabetes especially those with glycosuria, ketoacidosis, and excess urinary magnesium losses. Increased deficiency of magnesium potentially causes states of insulin resistance. Taking magnesium supplements improves the action of insulin and decrease blood sugar levels, particularly in the elderly [40]. Potassium supplementation yields improved insulin sensitivity, responsiveness and secretion in diabetics [43,44].

The intrapersonal administration of the aqueous stem bark extracts of *Aloe secundiflora* caused decrease in growth rate compared to the control mice. This could be caused by the toxic effect of the phytochemicals present in the extract. Synephrines (Alkaloid) cause reduction in body weight by increasing energy expenditure and decreasing feed intake and gastric motility [45]. Catechins (flavonoids) are reported to decrease glucose absorption through inhibition of small-intestine micelle formation and the inhibition of α -glucosidase activity. The decreased carbohydrate absorption leads to increased oxidation of body's fat reserves and causes weight loss. They are also reported to increase sympathetic nervous system activity and thermogenesis [45].

Tannins are astringent. Astringency is the sensation caused by the formation of complexes between tannins and salivary glycoproteins. This sensation causes decrease in palatability which in turn suppresses feed intake. Furthermore tannic acids are reported to cause hemorrhagic gastroenteritis which decreases absorption of nutrients. This negatively influences intake because of the filling effect associated with undigested food [46].

Proanthocyanidins (condensed tannins) are found to damage the mucosa of the gastrointestinal tract, decreasing the absorption of nutrients such as proteins, carbohydrates and essential amino acids such as methionine and lysine [47].

The investigated hematological parameters in this study are used as markers for the potential hemato-toxic effect of the extract in mammals and human beings. They provide information about the status of bone marrow activity and hemolysis [48]. Daily oral administration of 1 g/kg body weight of the extract caused decrease in platelet levels compared to control group. It is possible that some of the plant constituents may be toxic to thrombocytes directly or they depress thrombocyte production. Although rare, severe reduction in platelets count is associated with the risk of internal bleeding which can be fatal [49]. The mild increase in levels of MCHC resulted from daily intraperitoneal administration of 1 g/kg body weight of the extract could not be explained in the scope of this study.

Abnormalities in levels of creatine kinase, alanine transaminase, aspartate aminotransferase, alkaline phosphatase, γ -glutamyl transpeptidase, α -amylase, total bilirubin, direct bilirubin and creatinine could be due to tissue damage caused by toxic constituents

of the plant extract. These include among others, alkaloids, saponins, tannins and flavonoids

Saponins hemolyse red blood cells and cause cell death of many tissues [50,51]. In the kidneys, saponins lead to haemorrhage in the glomeruli and focal destruction of the renal tubules. Toxic levels of saponins cause cardiac failure, acute hypoglycemia and hepatorenal damage leading to death [51]. Alkaloids have been reported to cause liver megalocytosis, proliferation of biliary tract epithelium, liver cirrhosis and nodular hyperplasia [52]. Moreover tannins have been associated with severe central hepatonecrosis [53]. 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 1 g/kg body weight were below the recommended daily allowance.

Conclusion

The aqueous stem bark extracts of *Aloe secundiflora* 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 a 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 plants may have resulted from its phytochemical and mineral constituents.

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Conflict of interest

The authors declare no conflict of interest

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