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# *In vivo* antidiabetic activity of aqueous and ethyl acetate leaf extract of *Senna singuena* (Delile) in alloxan induced diabetic mice

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## ABSTRACT

The folkloric claims that *Senna singuena* confers antidiabetic effect to prescribed patients has received long term clinical application accompanied by limited scientific data in support of such claims. This study aimed at bioscreening for hypoglycemic activity of the aqueous and organic fractions of *S. singuena* in alloxan induced diabetic mice. Type I diabetes mellitus was induced in mice by intraperitoneal administration of alloxan monohydrate followed by graded doses of the aqueous and ethyl acetate leaf extract administered to the experimentally diabetic mice following an overnight fast. The composition of the various phytochemicals of the plant extract was quantitatively assessed using standard procedures. Oral and intraperitoneal administration of the aqueous and ethyl acetate leaf extract caused a significant reduction in plasma glucose level in a dose independent manner in both fractions. The hypoglycemic activity could be attributed to phytoconstituents found in the plant extract. The generated data supports the folkloric claims associating *S. singuena* with hypoglycemic effects. However, there is need for further studies on this plant to investigate the mechanism of its activity and determine its safety profiles in order to explore possibilities of developing a new antidiabetic drug.

**Keywords:** Diabetes mellitus, Alloxan monohydrate, Oral and intraperitoneal, Antidiabetic, hypoglycemic, *Senna singuena.*

## INTRODUCTION

Diabetes mellitus is a chronic endocrinological disorder characterized by high blood levels of glucose caused by insufficient secretion of insulin by the pancreas or improper utilization of insulin by target cells [1]. The incidence of diabetes mellitus (DM) has reached epidemic proportions attaining a global incidence of 171 million people in the year 2000 [2, 3] and this figure is projected to rise to 366 million by the year 2030 [4]. In Kenya, it is estimated that 190,400 people in the 20-79 age group suffer from diabetes mellitus [5].

The principle complications of diabetes mellitus are retinopathy, neuropathy, nephropathy and angiopathy, susceptibility to infections, hyperlipidemia and ketoacidosis [1, 6, 7]. These complications result in increased disability, reduced life expectancy and enormous health cost for virtually every society [6]. Diabetes is a costly disease, not only for the affected individual and his/her family, but also for the health authorities owing to its chronic nature, the severity of its complications and the means required to control them [8].

The major non-pharmacological therapeutic option for patients suffering from diabetes mellitus is lifestyle management [6]. Besides exercise, weight control and medical nutrition therapy, oral glucose-lowering drugs and insulin injection are the conventional therapies for the disease [8]. These conventional therapies have adverse side effects, are expensive and require expertise. Herbal medicines are cheap, more readily available to people and are assumed to be less toxic due to their long term clinical experience. However, a lot needs to be done to establish their efficacy and safety profiles, which in common practice is presumed from historical traditional use [9]. Among the plants traditionally used in the management of diabetes mellitus is *Senna singuena.*

*Senna singuena* (Delile) is a deciduous shrub 15m tall with 5–12 pairs of leaflets that belongs to the family Caesalpiniaceae [10]. It is widespread across the tropical Africa. It is been traditionally applied as therapy for fever, malaria, conjunctivitis, impotence, stomach upset, abdominal pain and diabetes [10, 11]. This study, therefore, sought to bio-screen *Senna singuena* used by traditional medicine practitioners in Kenya for their hypoglycemic activity and phytochemicals present in this plant in order to provide scientific data that can act as a proper guide for use of this plant in management of diabetic mellitus.

**Figure 1:** *Senna senguena* (photograph taken in July 2014, in Nthawa ward, Siakago Division, Mbeere North Sub-County, Embu County, Kenya)

## MATERIALS AND METHODS

### Study site

This study was undertaken at the Department of Biochemistry and Biotechnology, School of Pure and Applied Sciences, Kenyatta University.

### Collection of the plant materials and preparation of the extracts

The plant used in this study was collected from its natural habitat in Nthawa ward, Siakago Division, Mbeere North Sub-County, Embu County, Kenya. A taxonomist authenticated its botanical identity and voucher specimen deposited at the National Museums of Kenya Herbarium, Nairobi, Kenya for future reference. The collected leaves of *Senna singuena* were air-dried until completely dry then ground into fine powder using an electric mill.

For aqueous extraction, 100 g of the powdered plant material was extracted in 1 liter of distilled water for 6 hours at 60°C. The plant was then decanted and filtered using filter papers under vacuum pump. The filtrate was finally freeze-dried for 48 hours. The freeze-dried powder was weighed and stored in airtight container at -20°C ready for bioassay.

For organic extraction, 500 g of powdered *S. singuena* leaves were soaked in a litre of ethyl acetate for 48hrs. The extract was decanted and filtered using muslin cloth. The semisolid mass was vacuum dried using a rotary evaporator at $77^0$ C to obtain a solid residue. The extract was refrigerated at $-20^0$ C until used for analysis.

### 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 dose intraperitoneal administration of 186.9 mg/kg body weight of a freshly prepared 10% alloxan monohydrate [12]. Forty-eight hours later, 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 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 1ml) (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 1ml 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 1ml physiological saline) (100 mg/kg body weight) in 0.1ml physiological saline; Group VI consisted of alloxan induced diabetic mice either intraperitoneally or orally administered with 5 mg extract (50 mg extract in 1ml physiological saline) (200 mg/kg body weight) in 0.1ml physiological saline; Group VII consisted of alloxan induced diabetic mice either intraperitoneally or orally administered with 7.5 mg extract (75 mg extract in 1ml 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. The same experimental design was adapted for organic fraction too.

### 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).

### Phytochemical analysis

The extract was analyzed for the following phytochemicals: total phenolics, saponins, tannins, flavonoids, alkaloids and phytonutrients; vitamins B1, B3, and C using assessed using standard procedures [10].

### Determination of the mineral content

Atomic Absorption Spectrophotometry (AAS) and Total reflection X-ray Fluorescence (TXRF) techniques were employed for mineral content analysis.

*Total reflection X-ray Fluorescence System (TXRF)*

In the TXRF technique, the x-rays excite atom immediately at the surface of the sample which in turn emit radiation characteristic to them in all directions. A detector placed above the sample senses the fluorescence X-rays and not the background scatter to a very high sensitivity level.

Preparation of the sample:

To one gram of each plant sample was added 10 ml of double distilled water to a vial in triplicate. As an internal standard, 20 µl of 1000 ppm gallium stock solution was added to each sample resulting to a concentration of 2 ppm Ga. The mixture was vortexed for one minute and aliquots of 10 µl of each sample drawn and pipetted into quartz carriers in triplicate. The samples were placed in the oven in order to evaporate the liquid.

Sample spectrum acquisition and quantitative analysis was carried out by use of Picofox TXRF Spectrometer. Each sample was irradiated for 1000 seconds. The spectrometer was perated at 50 KV and a current of 1000 µA. S2 PICOFOX software was used to evaluate the measured spectra based on the selected elements. The following formula was used to calculate the elemental concentration:

$$Cis = \frac{N_\chi / S_\chi}{Nis / Sis} \, {}_\chi Cis$$

Where,

Cχ Concentration of the analyte; Cis Concentration of the internal standard

Nχ Net intensity of the analyte; Nis Net intensity of the internal standard

Sχ Relative sensitivity of analyte; Sis Relative sensitivity of internal standard

Raw data tabulated on excel worksheet was processed in order to arrive at the average and standard deviation for each sample. Final values were expressed as µg/g ppm.

*Atomic Absorption Spectrophotometry (AAS)*

The quantities of magnesium, chromium and vanadium in the plant samples were determined using Atomin Absorption Spectrophotometry (AAS). This method determines the quantity of the chemical elements by the absorption of optical radiation generated by the free atoms in the gaseous state. Standards with known analyte content are required to establish the correlation between the absorbance of the sample and the concentration of the analyte. This technique is based on the Beer-Lambert Law.

In the preparation of the wet digests for analysis, 1 g of the dry samples from each plant were put into 100 ml pyrex beakers to which was added 10 ml of concentrated nitric acid. The mixture was allowed to stand in order to soak. Subsequently, 3 ml of perchloric acid (60% $HClO_4$) was added to each beaker and the mixture slowly warmed on a hot plate until frothing ceased. This was followed by intense heating in order to evaporate all the nitric acid. After charring and cooling, 10 ml of concentrated nitric acid was further added and heating resumed until white fumes due to $HClO_4$ were observed. Filtration of the solution into 100 ml volumetric flasks was carried out using Whatman filter paper No 1. After vigorous shaking, the solution was transferred into plastic bottles and the samples kept in the freezer awaiting analysis.

Standard solutions for each element were prepared varied range (1ppm, 5ppm, 10ppm, 15ppm, 20ppm, and 25ppm) in order to give a linear relationship between concentration and absorbance. Aliquots of standard stock solution were drown and put into a series of flasks and diluted using distilled water to give the desired final concentration. For magnesium 2 ml of 5% Lanthum solution was added before dilution. Blank was prepared by adding all the reagents except the sample.

After setting up the AAS instrument, the respective standards and samples were aspirated into the flame in order to determine the absorbance. Absorbance at zero concentration was re-established by flashing distilled water into the flame. A calibration curve was plotted of the absorbance against the concentration of the standard solution. The above procedure for each sample was repeated in duplicate. The mean absorbance was determined and recorded. Final values were expressed as µg/g dry matter.

**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 ± Standard Deviation (SD). 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. $p \leq 0.05$ was considered statistically significant.

## RESULTS

**Effect of ethyl acetate leaf extract of *Senna singuena* administered intraperitoneally and orally in mice models**

In the induction of diabetes in mice models, alloxan caused significant elevation of the blood sugar levels. Analysis of the levels of the blood sugar indicated that it was only elevated at the 24$^{th}$ hour when compared to the 2$^{nd}$ hour ($p<0.05$). Administration of insulin to the diabetic mice models caused significant lowering of the blood sugar when compare to either the 0 hour for the 6$^{th}$, 8$^{th}$ and 24$^{th}$ hour ($p<0.05$). Administration of the various doses of the extracts caused significant lowering of the blood sugar levels in the same manner as those administered with insulin from the 2$^{nd}$ hour to the 8$^{th}$ hour followed with an elevation of the blood glucose level at the 24$^{th}$ hour (Figure 2). Administration of the 50 mg/kg body weight of the *Senna singuena* caused selective significant variation when compared to both 0 hour and 2$^{nd}$ hour respectively ($p<0.05$). The effects at a dose of 100 mg/kg body weight were similar to that of a dose 50 mg/kg of the extract. It was also observed that the effects generated at both doses of 200 and 300 mg/kg body weights of *S. singuena* were also same; they were significantly different at all hours in the same manner ($p<0.05$) (Table 1).

Oral administration of Glibenclamide caused a significant lowering of the blood glucose levels when compared to the alloxan diabetic groups of mice. An almost similar effect was observed when the organic leaves extracts of *S. singuena* were administered orally in the

mice models at the various doses of 50. 100, 200 and 300 mg/kg body weight when compared to the normal mice. However, various effects of the extracts were observed at the various doses of the extracts and at different hours post administration (Figure 3). The effects of insulin was only significant at the 8th hour and 24th hour respectively when compared to 0 hour and 2nd hour respectively ($p<0.05$). Administration of Glibenclamide was also significantly lower when compared to the 4th, 6th, 8th and 24th hour respectively ($p<0.05$). Administration of a dose of 50 mg/kg organic leave extract of *S. senguena* induced a significant reduction of blood glucose levels in alloxan induced diabetic mice at the 6th and 8th hour respectively ($p<0.05$). Administration of a dose of 100 mg/kg of *Senna senguena* at 4th, 6th and 8th induced significantly lower levels of blood sugar when compared to either 0 hour and 2nd hour or both ($p<0.05$). A dose of 200mg/kg of *S. senguena* caused significant low values in blood sugar levels at the 6th, 8th and 24th hour when compared to the zero hour and only at the 8th hour when compared to the 8th hour respectively ($p<0.05$). Administration of a dose of 200 mg/kg body weight also caused significantly lower levels of blood glucose levels at the 6th, 8th and 24th hour respectively ($p<0.05$). Finally, a dose of 300 mg/kg body weight caused significant low values at the 6th, 8th and 24th hour when compared to the 0 hour,2nd hour and 8th hour respectively ($p<0.05$) (Table 2).

**Table 1:** Effect of intraperitoneal administration of ethyl acetate leaf extract of *Senna senguena* in alloxan induced diabetic mice

| Treatment Groups | 0 hr | 2 hr | 4 hr | 6 hr | 8 hr | 24hrs |
|---|---|---|---|---|---|---|
| **Normal Control** | $5.22\pm0.04^{b}$ | $5.22\pm0.07^{c}$ | $5.18\pm0.04^{c}$ | $5.20\pm0.03^{b}$ | $5.18\pm0.02^{b}$ | $5.14\pm0.04^{c}$ |
| **Diabetic control** | $15.96\pm1.68^{a}$ | $17.30\pm1.68^{a}$ | $18.74\pm1.72^{a}$ | $21.02\pm1.51^{a}$ | $22.56\pm1.57^{a}$ | $24.00\pm1.27^{aB}$ |
| **Reference group** | $16.84\pm2.13^{aB}$ | $7.00\pm0.49^{cA}$ | $6.06\pm0.29^{bcA}$ | $5.22\pm0.19^{bA}$ | $4.80\pm0.14^{bA}$ | $6.98\pm0.54^{bcA}$ |
| **50mg/kgbw** | $17.32\pm2.06^{a}$ | $12.28\pm1.76^{b}$ | $9.10\pm1.06^{bA}$ | $6.00\pm0.58^{bAB}$ | $4.34\pm0.20^{bAB}$ | $8.34\pm0.55^{bA}$ |
| **100mg/kgbw** | $17.18\pm2.03^{a}$ | $9.32\pm0.92^{bc}$ | $6.78\pm0.63^{bcA}$ | $4.78\pm0.42^{bAB}$ | $3.78\pm0.39^{bAB}$ | $6.74\pm0.46^{bcA}$ |
| **200mg/kgbw** | $15.26\pm2.11^{aB}$ | $8.52\pm0.84^{bcA}$ | $6.24\pm0.65^{bcA}$ | $4.58\pm0.36^{bAB}$ | $3.92\pm0.43^{bAB}$ | $6.36\pm0.64^{bcA}$ |
| **300mg/kgbw** | $15.72\pm1.57^{aB}$ | $8.22\pm0.69^{bcA}$ | $6.30\pm0.48^{bcA}$ | $4.24\pm0.35^{bAB}$ | $3.12\pm0.41^{bAB}$ | $6.38\pm0.59^{bcA}$ |

Values are expressed as Means ± SEM for five animals per group. Means within respective columns followed by similar lower case letters are not significantly different at $p \leq 0.05$; means within respective rows followed by similar upper case letters are not significantly different at $p \leq 0.05$ following ANOVA and Tukey's post hoc test for multiple comparison.

**Table 2:** Effect of oral administration of ethyl acetate leaf extract of *Senna senguena* in alloxan induced diabetic mice

| Treatment Groups | 0 hr | 2 hr | 4 hr | 6 hr | 8 hr | 24hrs |
|---|---|---|---|---|---|---|
| **Normal Control** | $5.08\pm0.05^{b}$ | $5.16\pm0.04^{b}$ | $5.24\pm0.02^{c}$ | $5.20\pm0.03^{c}$ | $5.18\pm0.07^{b}$ | $5.10\pm0.04^{c}$ |
| **Diabetic control** | $13.90\pm1.19^{a}$ | $15.48\pm1.17^{a}$ | $16.72\pm1.27^{a}$ | $18.26\pm1.20^{a}$ | $19.52\pm1.24^{aA}$ | $22.10\pm1.42^{aAB}$ |
| **Reference group** | $14.32\pm1.40^{a}$ | $11.50\pm1.07^{a}$ | $8.80\pm0.98^{bcA}$ | $6.38\pm0.38^{bcAB}$ | $4.94\pm0.12^{bAB}$ | $8.40\pm0.46^{bcA}$ |
| **50mg/kgbw** | $13.34\pm1.32^{a}$ | $11.94\pm1.46^{a}$ | $8.90\pm1.14^{bc}$ | $6.76\pm0.88^{bcAB}$ | $5.54\pm0.50^{bAB}$ | $9.96\pm0.97^{b}$ |
| **100mg/kgbw** | $14.40\pm1.34^{a}$ | $12.72\pm1.57^{a}$ | $9.28\pm0.98^{bcA}$ | $6.94\pm0.77^{bcAB}$ | $5.68\pm0.47^{bAB}$ | $9.66\pm1.19^{b}$ |
| **200mg/kgbw** | $12.72\pm1.24^{a}$ | $10.88\pm1.00^{a}$ | $9.20\pm0.89^{bc}$ | $8.14\pm0.72^{bcA}$ | $6.54\pm0.40^{bAB}$ | $7.94\pm0.34^{bcA}$ |
| **300mg/kgbw** | $13.84\pm1.43^{a}$ | $12.32\pm1.52^{a}$ | $9.90\pm0.92^{b}$ | $8.68\pm0.76^{bA}$ | $7.34\pm0.32^{bAB}$ | $8.28\pm0.89^{bcA}$ |

Values are expressed as Means ± SEM for five animals per group. Means within respective columns followed by similar lower case letters are not significantly different at $p \leq 0.05$; means within respective rows followed by similar upper case letters are not significantly different at $p \leq 0.05$ following ANOVA and Tukey's post hoc test for multiple comparison.

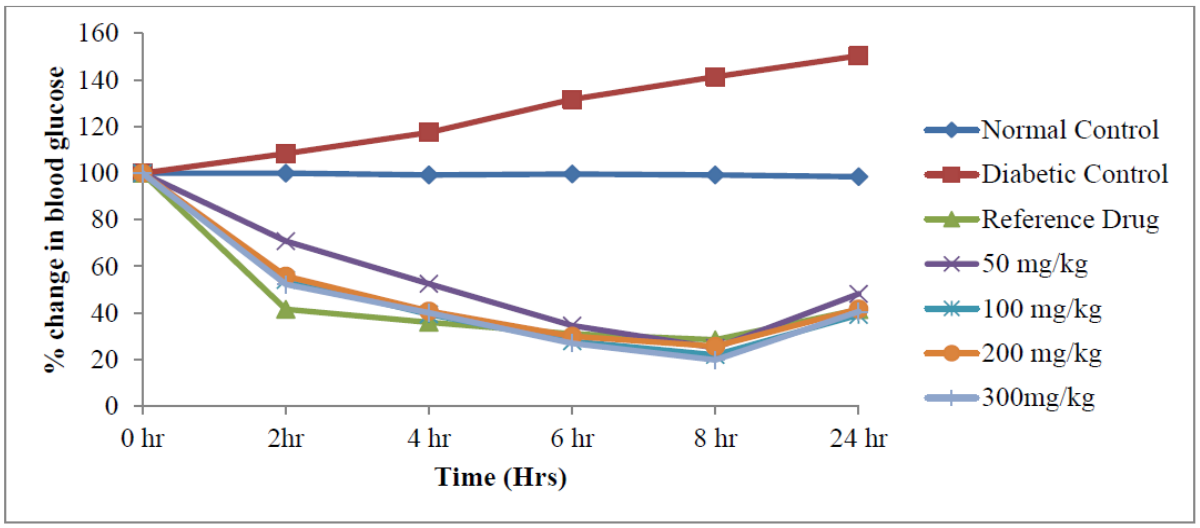


**Figure 2:** The mean percentage change in blood glucose levels after organic leaves extracts of *Senna singuena* was administered intraperitoneally in Alloxan induced diabetic mice.

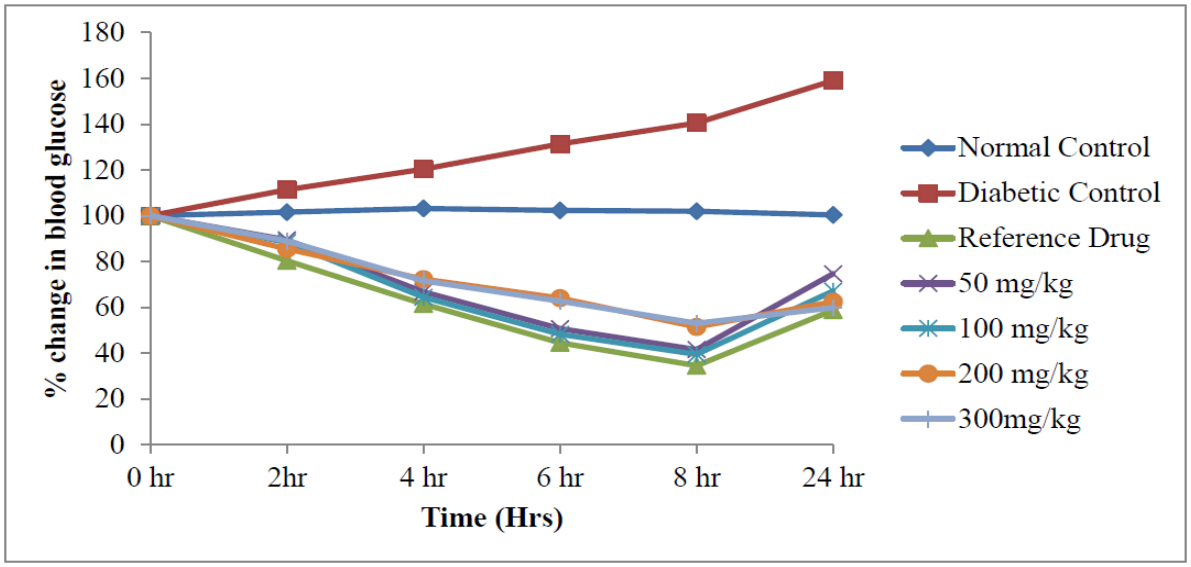


**Figure 3:** The mean percentage change in blood glucose levels after organic leaves extracts of *Senna singuena* was administered orally in Alloxan induced diabetic mice.

**Effect of aqueous leaf extract of *Senna singuena* administered intraperitoneally and orally in mice models**

Aqueous leaf extract of *S. singuena* at the various doses of the extract lowered the blood glucose levels in a similar way to the insulin treated mice when administered intraperitoneally. Diabetes was induced using alloxan and caused gradual elevation of the blood glucose levels from the 0 hour to the 24$^{th}$ hour respectively (Figure 4). It is only at the 24$^{th}$ hour that there was the blood glucose levels significantly elevated when compared to the 0 hour ($p<0.05$). In the reference group administered with insulin IP, all the groups were significantly different from the 0 hour and 2$^{nd}$ hour for the 0 hour ($p<0.05$). The effects induced by administration of dose of 50 mg/kg bodyweight of *S. singuena* were very similar to the effects of a dose of 100 mg/kg body weight of *S. singuena*. They all were significantly different from either the 0 hour or 2$^{nd}$ hour respectively ($p<0.05$). Administration of a dose of 200 mg/kg body weight of *S. singuena* induced significant variations at the 4$^{th}$, 6$^{th}$, 8$^{th}$ and 24$^{th}$ hour ($p<0.05$). Moreover, all the groups were also significantly different from 0 hour and 0 hour was significantly different from 2$^{nd}$ hour respectively ($p<0.05$) (Table 3).

The effects of the aqueous extracts of *S. singuena* when compared to orally administered Glibenclamide. The blood glucose levels increased gradually as time increased post administration of alloxan in mice (Figure 5). It is only at the 24 hour that a significant increase in the blood glucose levels was elevated compared to both 0 hour and 2$^{nd}$ hour ($p<0.05$). However, this was contrary with administration of the Glibenclamide which significantly lowered the blood glucose levels at the 4$^{th}$, 6$^{th}$, 8$^{th}$ and 24$^{th}$ hour when compared to 0 hour and 2$^{nd}$ hour respectively ($p<0.05$). The extracts of *S. singuena* induced a similar trend by lowering the blood glucose levels. At a dose of 50 mg/kg body weight, all the groups were lower than the 0 hour except at the 4$^{th}$, 6$^{th}$, 8$^{th}$ and 24$^{th}$ hour which were significantly lower ($p<0.05$). Similarly, a dose of 100 mg/kg of *S. singuena*, at all the doses were significantly different from the 0 hour ($p<0.05$). Only at the 6$^{th}$, 8$^{th}$ and 24$^{th}$ hour following the administration of a dose of 200 mg/kg body weight of *S. singuena* was significant variations observed when compared to the 0 hour ($p<0.05$). Finally, the effect of administration of a dose of 300 mg/kg which induced a similar effect compared to a dose of 100 mg/kg body weight ($p<0.05$) (Table 4).

**Table 3:** Effect of intraperitoneal administration of aqueous leaf extract of *Senna senguena* in alloxan induced diabetic mice

| Treatment Groups | 0 hr | 2 hr | 4 hr | 6 hr | 8 hr | 24hrs |
|---|---|---|---|---|---|---|
| **Normal Control** | $5.20\pm0.06^{b}$ | $5.20\pm0.07^{c}$ | 5.20±0.03+b | $5.22\pm0.02^{b}$ | $5.20\pm0.05^{b}$ | $5.14\pm0.05^{c}$ |
| **Diabetic control** | $14.64\pm1.66^{a}$ | $16.38\pm1.75^{a}$ | $17.70\pm1.51^{a}$ | $18.56\pm1.80^{a}$ | $20.14\pm1.64^{a}$ | $23.06\pm1.65^{aA}$ |
| **Reference group** | $14.36\pm1.10^{aB}$ | $7.22\pm0.28^{bcA}$ | $6.08\pm0.34^{bA}$ | $5.40\pm0.23^{bA}$ | $4.78\pm0.19^{bA}$ | $7.64\pm0.32^{bcADE}$ |
| **50mg/kgbw** | $15.24\pm1.24^{aB}$ | $11.06\pm1.34^{bA}$ | $8.08\pm0.62^{bA}$ | $6.14\pm0.40^{bAB}$ | $4.62\pm0.24^{bAB}$ | $9.98\pm0.72^{bA}$ |
| **100mg/kgbw** | $14.60\pm1.33^{aB}$ | $8.30\pm0.69^{bcA}$ | $6.34\pm0.56^{bA}$ | $4.88\pm0.33^{bAB}$ | $3.96\pm0.40^{bAB}$ | $7.78\pm0.46^{bcA}$ |
| **200mg/kgbw** | $13.36\pm1.58^{a}$ | $9.34\pm1.22^{bc}$ | $6.94\pm0.77^{bA}$ | $6.02\pm0.82^{bA}$ | $4.78\pm0.57^{bAB}$ | $8.88\pm0.53^{bA}$ |
| **300mg/kgbw** | $15.50\pm1.22^{aB}$ | $11.54\pm1.32^{abA}$ | $8.20\pm0.72^{bA}$ | $6.68\pm0.65^{bAB}$ | $5.38\pm0.40^{bAB}$ | $9.98\pm0.67^{bA}$ |

Values are expressed as Means ± SEM for five animals per group. Means within respective columns followed by similar lower case letters are not significantly different at $p \leq 0.05$; means within respective rows followed by similar upper case letters are not significantly different at $p \leq 0.05$ following ANOVA and Tukey's post *hoc* test for multiple comparison.

**Table 4:** Effect of oral administration of aqueous leaf extract of *Senna senguena* in alloxan induced diabetic mice

| Treatment Groups | 0 hr | 2 hr | 4 hr | 6 hr | 8 hr | 24hrs |
|---|---|---|---|---|---|---|
| **Normal Control** | 5.12±0.04$^{b}$ | 5.20±0.03$^{c}$ | 5.22±0.06$^{c}$ | 5.16±0.02$^{b}$ | 5.18±0.02$^{b}$ | 5.12±0.02$^{c}$ |
| **Diabetic control** | 13.82±1.42$^{a}$ | 15.06±1.48$^{a}$ | 16.66±1.59$^{a}$ | 18.10±1.58$^{a}$ | 19.48±1.50$^{a}$ | 22.00±1.18$^{aAB}$ |
| **Reference group** | 13.30±1.03$^{a}$ | 10.30±1.16$^{ab}$ | 8.42±0.81$^{bcA}$ | 6.82±0.52$^{bAB}$ | 5.54±0.30$^{bAB}$ | 7.80±0.37$^{bcA}$ |
| **50mg/kgbw** | 14.86±1.31$^{a}$ | 11.22±1.25$^{ab}$ | 8.00±1.09$^{bcA}$ | 6.20±0.62$^{bAB}$ | 4.92±0.44$^{bAB}$ | 9.62±0.73$^{bAE}$ |
| **100mg/kgbw** | 13.38±0.97$^{aB}$ | 9.840±0.78$^{bcA}$ | 7.36±0.44$^{bcA}$ | 6.02±0.34$^{bAB}$ | 4.98±0.36$^{bAB}$ | 7.80±0.44$^{bcA}$ |
| **200mg/kgbw** | 13.56±1.17$^{a}$ | 11.56±1.30$^{ab}$ | 9.90±0.84$^{b}$ | 8.06±0.46$^{bA}$ | 6.48±0.34$^{bAB}$ | 9.10±0.48$^{bA}$ |
| **300mg/kgbw** | 14.40±0.9$^{a}$ | 12.00±0.97$^{ab}$ | 9.40±1.25$^{bcA}$ | 7.14±0.71$^{bAB}$ | 5.74±0.74$^{bAB}$ | 9.46±0.71$^{bA}$ |

Values are expressed as Means ± SEM for five animals per group. Means within respective columns followed by similar lower case letters are not significantly different at p ≤0.05; means within respective rows followed by similar upper case letters are not significantly different at p ≤0.05 following ANOVA and Tukey's post *hoc* test for multiple comparison.

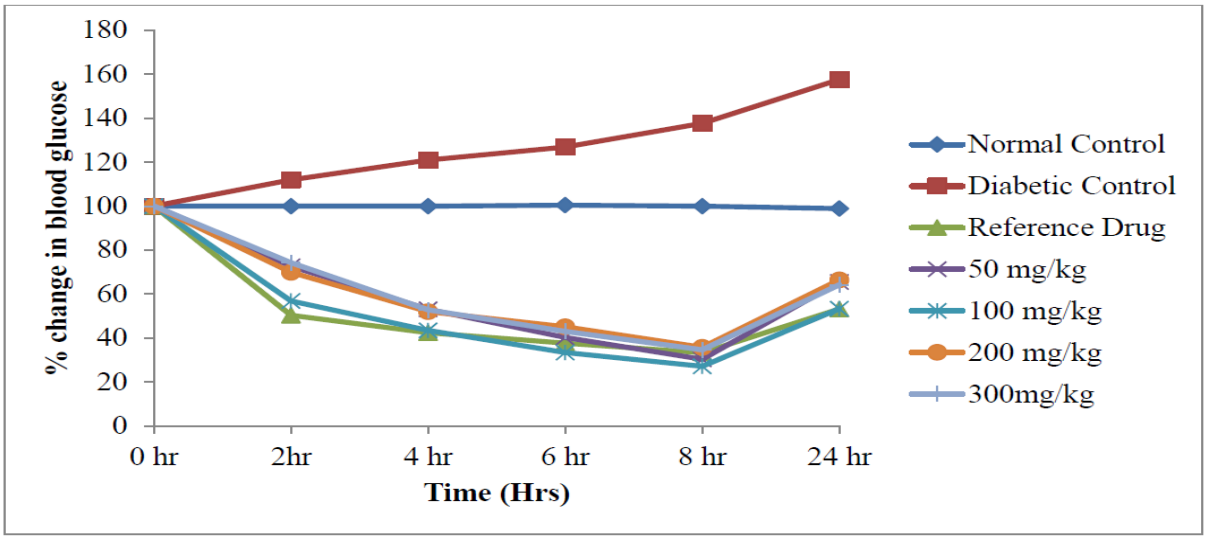


**Figure 4:** The mean percentage change in blood glucose levels after aqueous leaves extracts of *Senna singuena* was administered intraperitoneally in Alloxan induced diabetic mice.

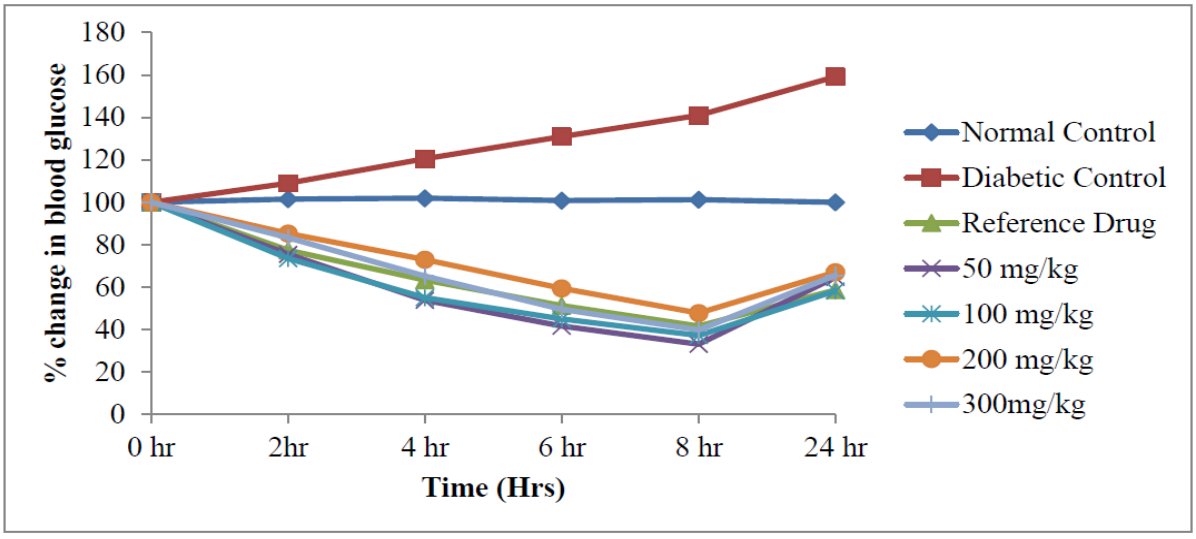


**Figure 5:** The mean percentage change in blood glucose levels after aqueous leaves extracts of *Senna singuena* was administered orally in Alloxan induced diabetic mice.

**Phytochemicals composition in *Senna singuena***

The phytochemicals detected in the leaf powder of *S. singuena* were total phenolics, saponins, tannins, flavonoids and alkaloids. The leaves extract were found to contain the following macronutrients in significant amounts; vitamin B1 and B3 and vitamin C (Table 5).

**Elemental analysis**

Various mineral elements were detected in the plant extract at different levels (Table 6). It is shown that Calcium, Potassium, iron, Manganese, Titanium were determined to be in high concentrations when compared to other mineral elements with concentrations of 40

ppm and above. However, calcium had the highest concentrations of more than 10,000 ppm when compared to the mineral elements. This was followed closely by potassium with concentrations of between 4,000 -9,000 ppm consequently followed by Titanium, Zinc and Manganese which had levels of approximately between 40 to 100 ppm.

Levels of less than 40 ppm were found in all the other minerals which included: Chromium, Vanadium, Nickel, Copper, Arsenic, Bromine, Rubidium, Strontium, Yttrium and Lead. Among these elements; radioactive elements were also present such as Strontium. Toxic elements such as Lead, Arsenic, and Nickel were also detected; however, they are in very low concentrations of below 40 ppm.

**Table 5:** Quantitative phytochemical analysis of the leaf powder

| **Quantity of phytochemicals in *Senna singuena*** | |
|---|---|
| **Type of the phytobiotic** | **Quantity** |
| Vitamin C | 3.52 (mg/100g) |
| Vit B1 | 0.45 (mg/100g) |
| Vit B3 | 0.21 (mg/100g) |
| % Alkaloid | 3.68% |
| T. Phenol | 35.99 (mg/100g) |
| Tannins | 2.67 (mg/100g) |
| Saponins (%) | 3.68% |
| Flavonoids | 75.51 (mg/100g) |

**Table 6:** Quantitative mineral analysis for the plant extract in µg/g (ppm)

| **Mineral content in *Senna singuena*** | |
|---|---|
| **Mineral element** | **Quantity in µg/g (ppm)** |
| Potassium (K) | 8900 ± 268 |
| Calcium (Ca) | 16128 ± 1520 |
| Titanium (Ti) | 92.3 ± 4.4 |
| Vanadium (V) | 14.2 ± 2.4 |
| Chromium (Cr) | <0.03 |
| Manganese (Mn) | 58.1 ± 2.6 |
| Iron (Fe) | 357 ± 5 |
| Nickel (Ni) | 10.4 ± 0.2 |
| Copper (Cu) | 8.10 ± 0.57 |
| Zinc (Zn) | 64.8 ± 0.4 |
| Arsenic (As) | 2.38 ± 0.42 |
| Bromine (Br) | 0.982 ± 126 |
| Rubidium (Rb) | 2.14 ± 0.18 |
| Strontium (Sr) | 153 ± 2 |
| Yttrium (Y) | <0.01 |
| Lead (Pb) | 16.9 ± 1.4 |

## DISCUSSION

The aim of this study was to investigate the *in vivo* antidiabetic effect of the aqueous and ethyl acetate leaf extract of *Senna singuena* in alloxan induced diabetic mice. The administration of alloxan monohydrate resulted in 3 to 4 times increase in plasma blood glucose levels compared to normal control group. The alloxan confers its toxicological effects by selective necrosis of pancreatic islet cells [13]. In both routes and fractions, administration of the plant extract showed hypoglycemic activity 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). Similar findings were obtained by Arika *et al*. [6] Karau *et al.* [14] and who reported that *Lippia javanica* and *Pappea capensis* showed hypoglycemic activity in alloxan induced diabetic mice respectively.

The possible mechanism for antidiabetic activity of *S. singuena* could be due to increased insulin secretion from regenerated or remnant beta cells or augmented stimulation of glucose uptake by the peripheral tissues [15].

This hypoglycemic activity could be due to the presence of phytobiotics that include, flavonoids, alkaloids, saponins, vitamin B and C, tannins and total phenols in the plant extract. Flavonoids have been shown to stimulate peripheral glucose uptake, regulate the activity and/or expression of the rate limiting enzymes, enhances lipogenesis, facilitate insulin release and conversion of proinsulin to insulin [16, 17].

Saponins from the seeds of *Entada phaseoloides* caused a significant decrease in fasting blood glucose levels in type 2 diabetic rats [18]. In addition, tannins and saponins were found to have hypoglycemic activity in elderly diabetic patients by inhibition of α-amylase and α-glucosidase enzymes [19, 20] and translocation of glucose transporter IV [21]. Vitamin B and C have associated with antidiabetic activity through their facilitated anti-oxidative capacity [22].

Isolated alkaloids from *Catharanthus roseus* increased glucose uptake in pancreatic and muscle cells via inhibition of protein tyrosine phosphatase PTP-1B (a down regulator in the insulin signaling pathway) [23].

The observed gradual elevation of blood glucose by the 24 hour could be due to short half-life of the extract or it could have undergone a fast hepatic metabolism or renal clearance [13].

The anti-diabetic acitivity of the plant extract could also be attributed to the presence of trace elements which have been found to confer hypoglycemic activity [24]. Zinc is involved in the regulation of insulin receptor-initiated signal transduction mechanisms and insulin receptor synthesis. It has enhances the effectiveness and release of insulin [25]. Pottasium is necessary for optimal insulin secretion. Potasium depletion can result in reduced glucose tolerance [25]. Vanadium was reported to mimic the metabolic effects of insulin in rat adipocytes Vanadium therapy was shown to normalize blood glucose levels in STZ induced rats and to cure many hyperglycemia related deficiencies [25]. Magnesium is a cofactor in the glucose-transporting mechanism of the cell membrane and various enzymes in carbohydrate oxidation [25]. Iron is mainly involved in oxidation reduction reactions, hemoglobin oxygen transport and also a cofactor for numerous other enzymes. Manganese supplements have reversed the impaired glucose utilization induced by manganese deficiency in guinea pigs. Copper is required for absorption and transport of iron and it plays a key role in hemoglobin synthesis [25].

## CONCLUSION

The aqueous and ethyl acetate leaf extract of *S. singuena* had antidiabetic activity. The phytochemical screening of aqueous and ethyl acetate leaf extract of *S. singuena* indicated the presence of phenols, alkaloids, flavonoids, tannins, saponins, and Vitamin B and C. Analysis of the mineral content revealed the presence of Potassium (K), Calcium (Ca), Titanium (Ti), Vanadium (V), Chromium (Cr), Manganese (Mn), Iron (Fe), Nickel (Ni), Copper (Cu), Zinc (Zn), Arsenic (As), Bromine (Br), Rubidium (Rb), Strontium (Sr), Yttrium (Y), Lead (Pb). The antidiabetic activity of the plant extract could be attributed to presence of the phytobiotics and various identified trace elements. This study, therefore, recommends use of aqueous and ethyl acetate leaf extract of *S. singuena* in management of diabetes mellitus. However, further studies need to be done isolate and identify specific constituents responsible for the bioactivity through bioassay guided fractionation.

**Conflict of interest**

Authors declare that there is no conflict of interest to reveal.

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