

# *In Vivo* Hypoglycemic Activity of Aqueous Extracts of *Chasmanthera dependens* in Alloxan Induced Diabetic Mice

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## Abstract

The plant *Chasmanthera dependens* (Hochst) have been used to manage many diseases including diabetes mellitus without scientific evaluation of its efficacy and safety. The main objective of the project was to determine the antidiabetic activity of *Chasmanthera dependens* plant in a rat model. Experimental design was used where the stem of the plant was collected, dried, crashed into fine powder, extracted with distilled water at 60°C and lyophilized using a Freeze Dryer, packaged in air tight container and stored at -20°C ready for use. The extract was orally and intraperitoneally screened in alloxan induced diabetic mice for its hypoglycemic activity at doses of 25, 48.4, 93.5, 180.9 and 350 mg per kg body weight. Diabetes in mice was induced using 186.9 mg/kg of alloxan monohydrate. Negative controls included normal and diabetic rats orally and intraperitoneally administered with physiological saline while positive controls included diabetic rats administered with glibenclamide as oral and insulin as intraperitoneal reference drug. The results revealed hypoglycemic activity in *Chasmanthera dependens* at the five different doses when given either orally or intraperitoneally. In conclusion, the medicinal plant at the various doses demonstrated significant ( $p \leq 0.05$ ) hypoglycemic activity. The author's recommendation is continued use of the plant extracts at low therapeutic doses. Consideration should be made to carry out the same studies using higher animals including man.

## Keywords

Diabetes, Hypoglycemia, Plant Extract, *Chasmanthera dependens*, Albino Mice

## 1. Introduction

Diabetes mellitus is a metabolic disorder with an underlying feature of hyperglycemia and disturbances in carbohydrate, protein and fat metabolism. The disease may present with characteristic symptoms such as polydipsia, polyuria, polyphagia, blurring of vision, and weight loss and in its most severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death [1]. During onset, symptoms may not be present and consequently hyperglycemia sufficient to cause pathological and functional changes may be present for a long time before the diagnosis is made. Hyperglycemia may be as a result of defects in insulin secretion, insulin action or both. The chronic state and attendant metabolic disturbances may be associated with secondary damage in multiple organ systems, especially the kidneys, eyes, nerves, and blood vessels which results to coronary heart disease and cerebrovascular disease [2].

Management of diabetes mellitus can be achieved through the oral antidiabetic agents like sulfonylureas, biguanides, meglitinide, D-phenylalanine derivatives, and alpha-glucosidase inhibitors among others or the intraperitoneal form, insulin. The use of oral antidiabetic drugs has adverse effects including hematological, cutaneous and gastrointestinal reactions, hypoglycemic coma, flatulent, diarrhea and impairment of liver and kidney functions [3]. Some do not cause low blood sugar when used alone and require combination therapy.

Plants have always been good source of drugs; ethnobotanical information reports that about 800 plants may possess anti-diabetic potential [4]. Several plants have been used as dietary adjuvant and in treating a number of diseases even without knowledge on their proper functions and composition. Metformin, a less toxic biguanides and a potent oral glucose-lowering agent, was developed from *Galega officianalis* and is used to treat diabetes [5]. Research has indeed revealed that aqueous stem bark extracts of some plants have potent hypoglycemic activity in alloxan-induced diabetic mice [6].

The resulting complications in Diabetes mellitus are life threatening and the increasing number of people suffering from the disease face a number of challenges on the current antidiabetic agents in terms of cost and side effects. *Chasmanthera dependens* is gaining popularity in Keiyo South (Kenya) in the management of diabetes mellitus as alternative medicine; however, its efficacy and long term safety has not been scientifically evaluated. The aim of the study was to determine the efficacy and safety of *Chasmanthera dependens* in the management of diabetes mellitus.

## 2. Materials and Methods

### 2.1. Collection and Preparation of the Plant Material

Plant parts were collected from Songita village, Kiptuilong location, Keiyo South Constituency in Elgeiyo Marakwet County, Kenya. The sample plants were dried in a cool dry place for four weeks after splitting into small pieces before grinding

into powder using an electric grinder. The powder was kept in a cool dry place at room temperature in dry plastic bags ready for extraction.

## 2.2. Extraction of Plant Material

100 g of the powder was extracted in one liter of distilled water at 60°C in a shaker for 6 hrs and thereafter left to cool to room temperature. The extract was filtered into clean dry conical flask through gauze then through Whatman filter papers under vacuum pressure. Filtration was repeated until the supernatant collected in each case was clear. The filtrate was then freeze dried in 200 ml portions using Freeze Dryer for 48 hrs then stored in air tight containers at -20°C ready for bioassay.

## 2.3. Preparation of Plant Extracts for Injection into Mice

The freeze-dried plant extracts was dissolved in 1 ml physiological saline to make five doses for injection into mice. 5.75 mg was dissolved to inject at 25 mg/kg, 11.13 mg at 48.4 mg/kg, 21.51 mg at 93.5 mg/kg, 41.607 mg at 180.9 mg/kg and 80.5 mg to inject at 350 mg/kg body weight. 0.1 ml of each plant extract solutions was administered either intraperitoneally or orally to different group sets of mice. Insulin dose was prepared by dissolving 2.5 insulin units in 1 ml physiological saline to inject at a 1 IU/kg body weight. Similarly, 46 mg of Glibenclamide was dissolved in 1 ml of physiological saline to administer orally at dose of 200 mg/kg body weight. The volume administered was 0.1 mls to each mouse.

## 2.4. Experimental Design

Swiss albino male mice of 4 - 6 weeks old and between 21 - 25 g were used for the study. The animals were bred in the Department of Biochemistry and Biotechnology, Kenyatta University animal house and were fed on a standard pellet diet and water. The mice were randomly grouped into four of five mice each 3 days before the start of the experiment. Groups II, III, and IV mice were injected with alloxan monohydrate at a dose of 186.9 mg/kg body weight to induce diabetes 72 hours before the start of the experiment. The mice in Group I was not given alloxan and acted as normal controls. Blood glucose levels were measured and a mouse with a value above 10.0 mmol/L was considered diabetic and was included in the experiment. Group I of five untreated mice was treated with 0.1 ml of physiological saline and represented the normal control experimental animals. Group II composed of five diabetic mice were treated with 0.1 ml of physiological saline representing the diabetic control. Group III of five diabetic mice represented the Standard control and was treated with insulin at a dose of 1 U/kg body weight for intraperitoneal route or 200 mg/kg body weight of Glibenclamide for the oral treatment. Group IV were treated with plant extracts at five dose levels each dose level comprising of five diabetic mice. The five dose levels were: 25 mg/kg body weight, 48.4 mg/kg body weight, 93.5 mg/kg body weight, 180.9 mg/kg body weight and 350 mg/kg body weight.

## 2.5. Blood Glucose Determination

Blood was obtained from the tails of the mice by snipping the tips with sterile scissors after swabbing with 70% ethanol. Bleeding was enhanced by gently milking the tail from the body towards the tip; blood squeezed into a soft style glucose strip and glucose level measured in mM using a soft style glucometer. Baseline blood glucose in all the mice was determined before treatment with Physiological saline, insulin or Glibenclamide or plant extracts. Hypoglycemic activity after treatment was analyzed by collecting blood at 0, 2, 4, 6, 8 and 24 hours for glucose determination and results recorded upon every collection. During the experiment the animals were allowed free access to water and food.

## 2.6. Plant Extract Phytochemical Analysis

The plant extracts was tested for secondary metabolites and chemical nutrients using standard methods.

## 2.7. Tests for Secondary Metabolites

Secondary metabolites including alkaloids, flavonoids, tannins, saponins and total phenols were analyzed using standard methods according to [7] with slight modification (Table 1).

## 2.8. Quantitative Determination of Saponins

1.0 g each of the samples was extracted with methanol in soxhlet apparatus for 8 hours. The methanolic extracts were evaporated under reduced pressure to afford crude methanolic which were partitioned between hexane and water in separating funnels. The aqueous layers were then extracted with diethyl ether. The aqueous layers were recovered while the diethyl ether layers were discarded and purification process repeated. The aqueous layers were further partitioned with *n*-butanol three times in each case. The combined butanol extracts were washed twice with 15 ml of 5% sodium chloride and the evaporated in *vacuo* to yield crude saponins whose contents were expressed as percentage.

## 2.9. Determination of Alkaloids

The alkaloid content was determined gravimetrically [8] with some modifications. 1.0 gm of each sample was first defatted three times using hexane followed by extraction using 50 ml of 10% acetic acid in ethanol. The mixture was shaken well, covered and allowed to stand for 4 hours. The mixtures were then filtered and the extracts concentrated in water bath until 0.50 of the original volume was attained. Concentrated ammonium hydroxide was then added drop wise in order to precipitate the alkaloids.

**Table 1.** Phytochemical content of the aqueous plant extracts of *C. dependens* (mg/g).

TANNINS	TOTAL PHENOLS	FLAVONOID	SAPONIN	ALKALOIDS
1.0 ± 0.1	0.5 ± 0.0	14.6 ± 0.6	67.1 ± 2.4	79.0 ± 1.5

Pre-weighed filter papers were used to filter off the precipitates which were then washed with 1% ammonium hydroxide solution. The filter papers containing the precipitates were dried in an oven at 60° for 30 minutes, transferred into a desiccator to cool and then reweighed until constant weights were obtained. The weights of the alkaloid were determined by weight differences of the filter paper. The experiments were replicated twice for each sample and the readings recorded as average of the two replicates.

### **2.10. Determination of Total Flavonoids**

The total flavonoid concentration was measured by the aluminum chloride colorimetric assay [9]. 0.15 gm of the extract was added to 4 ml of double distilled water in a 10 ml volumetric flask. To the above mixture, 0.3 ml of 5% NaNO<sub>2</sub> was added. After 5 minutes, 0.3 ml of 10% AlCl<sub>3</sub> was added. After 6 minutes, 2 ml of 1M NaOH was added and the total volume was made up to 10 ml with double distilled water. The solution was mixed well and absorbance was measured at 510 nm against a blank. The flavonoid content was determined using quercetin as standard.

### **2.11. Determination of Tannins**

The tannins were determined as follows; 2 gm of plant extract was extracted three times in 70% acetone. After centrifuging the sample supernatant was removed. Different aliquots were taken and final volume to 3 ml was adjusted by distilled water. The solution after vortexing were mixed with 1 ml of 0.016M K<sub>3</sub>Fe (CN)<sub>6</sub>, followed by 1 ml of 0.02M FeCl<sub>3</sub> in 0.1M HCL. Vortexing was repeated and the tubes were kept as such for 15 minutes. 5 ML of stabilizer (3:1:1 ratio of water, H<sub>3</sub>PO<sub>4</sub> and 1% gum Arabic) was added followed by revortexing. Absorbance was measured at 700 nm against blank. A standard curve was plotted using various concentrations of 0.001M garlic acid [10].

### **2.12. Determination of Total Phenols**

The total phenolic content was determined using Folin-Ciocalteu reagent and garlic acid as the standard according to the method by [11]. About 500 mg of plant extract was weighed and homogenized in 10 ml of aqueous acetone (70%). The homogenate was centrifuged at 10,000 rpm for 20 minutes and the supernatant was used in determination of phenol as follows; 0.5 ml of Folin-Ciocalteu 2N reagent was added to 2.5 ml of the supernatant and then 2 ml of 10% sodium carbonate in ethanol. The mixture was incubated for 5 minutes at 20°C and the absorbance read in triplicates at a wavelength of 710 nm.

### **2.13. Determination of Phytonutrients**

The different trace elements present in the plant extracts were determined using Energy Dispersive X-ray Fluorescence (EDXRF) analytical technique and Atomic Absorption Spectrophotometer (AAS).

### 2.14. Elemental Analysis by Total Reflection X-Ray Fluorescence System (TXRF System)

TXRF system was used to determine the content of Sodium (Na), Magnesium (Mg), 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 [9]. Samples were prepared by weighing three sets of 1 gram each of the lyophilized sample into clean vials. 10 ml of double distilled water was added to each sample for dissolution. 20  $\mu\text{L}$  of 1000 ppm gallium stock solution was added into each sample (as internal standard) resulting into a concentration of 2 ppm Ga in each sample. Each sample was homogenized for 1 minute using a vortex mixer. Aliquots of 10  $\mu\text{L}$  of each sample were pipetted out using a micropipette onto a clean quartz carrier. The carriers were then dried in an oven to evaporate the liquid. The main principle of X-ray Fluorescence Spectroscopy (XRF) is that when atoms are irradiated with X-rays, they emit secondary X-rays called fluorescence radiation. These fluorescence radiations are characteristic for a particular atom (element) and are of specific energy which makes it possible for qualitative and quantitative analyses.

Each sample carrier was irradiated for 1000 seconds using a S2 PICOFOX TXRF Spectrometer which was operated at 50 kV and a current of 1000  $\mu\text{A}$  [12]. The spectrometer uses a molybdenum anode. Evaluation of the measured spectra was done using S2 PICOFOX software on the basis of the chosen elements (Table 2). Using the same software (S2 PICOFOX), concentrations were calculated based on the net intensities of the element peaks as per the following formula;

$$C_x = \frac{N_x/S_x}{N_{is}/S_{is}} \times C_{is}$$

where,

- $C_x$ -Concentration of the analyte;
- $C_{is}$ -Concentration of the internal standard;
- $N_x$ -Net intensity of the analyte;
- $N_{is}$ -Net intensity of the internal standard;
- $S_x$ -Relative sensitivity of analyte;
- $S_{is}$ -Relative sensitivity of internal standard.

### 3. Results

Oral administration of aqueous extracts of *Chasmanthera dependens* at doses of 48.4, 93.5, 180.9, and 350 mg/kg body weight to alloxan induced diabetic mice lowered blood glucose to levels similar to those of the reference drug, glibenclamide from the second to the six hour. However, this reduction in blood glucose in alloxan induced diabetic mice within the same time periods was statistically higher than that of aqueous extracts of *Chasmanthera dependens* at 25 mg/kg

**Table 2.** Mineral Elements Analysis and the amount given to each mouse from the aqueous extract of *C. dependens* (ppb).

ELEMENT	<i>C. dependens</i>	RDA for mice ( $\mu\text{g}/\text{day}$ )
<b>K</b>	7,871,865 $\pm$ 245,645	
450 mg/kgbw	81.5	$3.5 \times 10^6$ (1250)
670 mg/kgbw	121.3	
1000 mg/kgbw	181.1	
<b>Ca</b>	614,592 $\pm$ 19,765	
450 mg/kgbw	6.4	$1.0 \times 10^6$ (357.1)
670 mg/kgbw	9.5	
1000 mg/kgbw	14.1	
<b>Ti</b>	<280	
450 mg/kgbw	0.0	
670 mg/kgbw	0.0	
1000 mg/kgbw	0.0	
<b>V</b>	655 $\pm$ 52	
450 mg/kgbw	0.0	$<1.8 \times 10^3$ (0.64)
670 mg/kgbw	0.0	
1000 mg/kgbw	0.0	
<b>Cr</b>	<150	
450 mg/kgbw	0.0	$3.5 \times 10$ (12.5)
670 mg/kgbw	0.0	
1000 mg/kgbw	0.0	
<b>Mn</b>	3809 $\pm$ 173	
450 mg/kgbw	0.0	$2.3 \times 10^3$ (0.82)
670 mg/kgbw	0.1	
1000 mg/kgbw	0.1	
<b>Fe</b>	5098 $\pm$ 208	
450 mg/kgbw	0.1	$8.0 \times 10^3$ (2.9)
670 mg/kgbw	0.1	
1000 mg/kgbw	0.1	
<b>Cu</b>	685 $\pm$ 43	
450 mg/kgbw	0.0	$1.5 \times 10^3$ (0.54)
670 mg/kgbw	0.0	
1000 mg/kgbw	0.0	
<b>Zn</b>	3968 $\pm$ 151	
450 mg/kgbw	0.0	$1.1 \times 10^4$ (3.9)
670 mg/kgbw	0.1	
1000 mg/kgbw	0.1	
<b>As</b>	176 $\pm$ 15	
450 mg/kgbw	0.0	
670 mg/kgbw	0.0	

## Continued

1000 mg/kgbw	0.0	
<b>Se</b>	<40	
450 mg/kgbw	0.0	3.5 × 10 (0.0125)
670 mg/kgbw	0.0	
1000 mg/kgbw	0.0	
<b>Br</b>	4864 ± 174	
450 mg/kgbw	0.1	
670 mg/kgbw	0.1	
1000 mg/kgbw	0.1	
<b>Rb</b>	1857 ± 76	
450 mg/kgbw	0.0	
670 mg/kgbw	0.0	
1000 mg/kgbw	0.0	
<b>Sr</b>	6608 ± 233	
450 mg/kgbw	0.1	
670 mg/kgbw	0.1	
1000 mg/kgbw	0.2	
<b>Hg</b>	<100	
450 mg/kgbw	0.0	
670 mg/kgbw	0.0	
1000 mg/kgbw	0.0	
<b>Pb</b>	341 ± 29	
450 mg/kgbw	0.0	
670 mg/kgbw	0.0	
1000 mg/kgbw	0.0	

body weight dose ( $\rho < 0.05$ ). In the eighth hour, all the five extract doses including the reference drug, glibenclamide, had lowered blood glucose of alloxan induced diabetic mice by similar levels to those of the normal control mice ( $\rho > 0.05$ ). Thereafter, the blood glucose levels of the alloxan induced diabetic mice rose towards the diabetic state levels from the eighth hour to the twenty-fourth hour at similar levels for all the five extracts doses including the reference drug, glibenclamide ( $\rho > 0.05$ ) (Table 3; Figure 1).

In the second hour, intraperitoneal administration of aqueous extracts of *Chasmanthera dependens* at 25, 48.4, 93.5, 180.9, and 350 mg/kg body weight in alloxan induced diabetic mice lowered blood glucose to similar levels which were similar to those induced by the reference drug, insulin ( $\rho > 0.05$ ). This blood glucose level which was similar to that of the normal control mice induced by all the five extract doses including the reference drug, insulin was maintained up to the twenty-fourth hour ( $\rho > 0.05$ ) (Table 3; Figure 2).



**Table 3.** Effects of oral and intraperitoneal administration of aqueous extracts of *Chasmanthera dependens* at therapeutic doses on blood glucose levels in alloxan induced diabetic mice.

Treatment	Route	Glucose levels at different hours after treatment					
		0 hr	2 hrs	4 hrs	6 hrs	8 hrs	24 hrs
Normal control	Oral	5.3 ± 0.2 <sup>Aa</sup>	5.2 ± 0.1 <sup>Aa</sup>	5.3 ± 0.1 <sup>Aa</sup>	5.2 ± 0.1 <sup>Aa</sup>	5.2 ± 0.1 <sup>Aa</sup>	5.2 ± 0.1 <sup>Aa</sup>
	IP	5.2 ± 0.1 <sup>Aa</sup>	5.2 ± 0.1 <sup>Aa</sup>	5.1 ± 0.1 <sup>Aa</sup>	5.1 ± 0.1 <sup>Aa</sup>	5.0 ± 0.2 <sup>Aa</sup>	5.1 ± 0.1 <sup>ABa</sup>
Diabetic plus Saline	Oral	15.5 ± 5.2 <sup>Ca</sup>	17.3 ± 5.4 <sup>Ba</sup>	18.5 ± 5.5 <sup>Ca</sup>	19.8 ± 5.0 <sup>Ba</sup>	21.4 ± 4.2 <sup>Ba</sup>	22.8 ± 4.1 <sup>Ca</sup>
	IP	12.6 ± 1.8 <sup>BCa</sup>	14.0 ± 1.4 <sup>Bab</sup>	15.4 ± 1.4 <sup>Babc</sup>	17.0 ± 1.4 <sup>Bbc</sup>	20.7 ± 2.4 <sup>Bcd</sup>	18.5 ± 1.8 <sup>Cd</sup>
Diabetic plus Gliben/insulin	Oral	15.2 ± 6.1 <sup>Cb</sup>	11.5 ± 5.0 <sup>ABab</sup>	10.1 ± 4.4 <sup>ABab</sup>	8.0 ± 2.9 <sup>Aab</sup>	5.7 ± 1.0 <sup>Aa</sup>	7.4 ± 0.8 <sup>ABa</sup>
	IP	13.7 ± 2.9 <sup>Cb</sup>	5.6 ± 0.7 <sup>Aa</sup>	4.9 ± 0.3 <sup>Aa*</sup>	4.8 ± 0.3 <sup>Aa</sup>	7.0 ± 1.1 <sup>Aa</sup>	4.7 ± 0.3 <sup>Aa</sup>
<u>Extract dose (mg/kg body weight)</u>							
25	Oral	17.1 ± 4.5 <sup>Cb</sup>	14.8 ± 3.6 <sup>Bab</sup>	12.8 ± 3.7 <sup>BCab</sup>	11.4 ± 3.8 <sup>Aab</sup>	8.7 ± 2.3 <sup>Aa</sup>	10.6 ± 2.5 <sup>Bab</sup>
	IP	9.4 ± 0.7 <sup>Bb*</sup>	4.0 ± 0.5 <sup>Aa*</sup>	3.6 ± 0.8 <sup>Aa*</sup>	3.8 ± 1.2 <sup>Aa*</sup>	3.6 ± 0.2 <sup>Aa*</sup>	3.4 ± 0.2 <sup>Aa*</sup>
48.4	Oral	15.2 ± 3.9 <sup>Cb</sup>	12.2 ± 4.2 <sup>ABab</sup>	9.5 ± 3.1 <sup>ABab</sup>	8.3 ± 3.7 <sup>Aab</sup>	6.5 ± 1.8 <sup>Aa</sup>	10.2 ± 2.7 <sup>Bab</sup>
	IP	11.8 ± 0.6 <sup>BCb</sup>	4.1 ± 0.6 <sup>Aa*</sup>	4.0 ± 0.8 <sup>Aa*</sup>	3.8 ± 1.0 <sup>Aa</sup>	4.5 ± 1.2 <sup>Aa</sup>	3.5 ± 0.7 <sup>Aa*</sup>
93.5	Oral	14.9 ± 5.8 <sup>ABb</sup>	11.7 ± 4.9 <sup>ABab</sup>	8.5 ± 3.9 <sup>ABab</sup>	6.8 ± 0.5 <sup>Aa</sup>	5.1 ± 1.0 <sup>Aa</sup>	10.2 ± 1.5 <sup>Bab</sup>
	IP	12.8 ± 1.1 <sup>BCb</sup>	5.4 ± 1.1 <sup>Aa*</sup>	4.5 ± 0.7 <sup>Aa</sup>	4.2 ± 0.7 <sup>Aa</sup>	4.0 ± 1.2 <sup>Aa</sup>	4.3 ± 0.9 <sup>Aa*</sup>
180.9	Oral	13.6 ± 3.3 <sup>ABc</sup>	10.8 ± 2.3 <sup>ABbc</sup>	8.8 ± 1.5 <sup>ABab</sup>	7.4 ± 0.7 <sup>Aa</sup>	5.6 ± 1.1 <sup>Aa</sup>	8.7 ± 1.8 <sup>ABab</sup>
	IP	12.5 ± 3.5 <sup>BCb</sup>	7.0 ± 6.3 <sup>Aa</sup>	4.2 ± 1.0 <sup>Aa*</sup>	3.8 ± 0.9 <sup>Aa*</sup>	3.7 ± 1.2 <sup>Aa*</sup>	3.3 ± 1.0 <sup>Aa*</sup>
350	Oral	16.9 ± 4.1 <sup>Cc</sup>	14.2 ± 2.9 <sup>Bbc</sup>	11.8 ± 2.3 <sup>ABCbc</sup>	9.1 ± 3.5 <sup>Aab</sup>	6.1 ± 1.4 <sup>Aa</sup>	9.9 ± 1.9 <sup>ABab</sup>
	IP	13.9 ± 2.6 <sup>Cb</sup>	5.6 ± 2.5 <sup>Aa*</sup>	4.3 ± 2.3 <sup>Aa*</sup>	3.8 ± 1.1 <sup>Aa*</sup>	3.6 ± 1.9 <sup>Aa*</sup>	4.2 ± 2.1 <sup>Aa*</sup>

Results are expressed as Mean ± SD for five animals per group. Values in respective columns followed by similar upper case letters across treatments and values in respective rows followed by similar lower case letters are not significantly different at  $\rho \leq 0.05$  analyzed by ANOVA followed by Tukey's post hoc test. Values before an\* are significantly different in route of administration analyzed by student T test.

Oral administration of all the five aqueous extract doses of *Chasmanthera dependens* in alloxan induced diabetic mice significantly reduced blood glucose to levels lower than those induced by the same five extract doses intraperitoneally administered to alloxan induced diabetic mice at second, fourth, sixth and the twenty-fourth hour ( $\rho < 0.05$ ). At the eighth hour, oral administration of aqueous extracts of the five doses of *Chasmanthera dependens* in alloxan induced diabetic mice reduced blood glucose to levels similar to those attained by the five aqueous extract doses of *Chasmanthera dependens* administered intraperitoneally ( $\rho > 0.05$ ).

Concentration of each mineral are expressed as  $\mu\text{g}/\text{kg}$  of dry powder of studied plant and are in the upper row; the amount of each mineral administered to mice in  $\mu\text{g}$  based on its concentration is in the lower row. This is compared with the recommended daily allowance shown in the last column and RDA in mice (in brackets). Recommended daily allowance is estimated from that of human beings stated in [13].

#### 4. Discussion

The induction of diabetes was done 72 hrs before the start of the experiment using alloxan monohydrate at a dose of 186.9 mg/kg body weight [14]. Thereafter, different groups of animals were subjected to the four sets of treatment (control normal, control diabetic, control diabetic on reference drug and diabetic on aqueous plant extracts). The oral and intraperitoneal administration of aqueous extracts of *Chasmanthera dependens* at all the five tested doses reduced blood glucose within the study period of twenty four hours with maximum activity at the eighth hour which was comparable to the effects of the reference drug (glibenclamide or insulin) in the model animals. This may be attributed to the presence of phytochemicals with hypoglycemic effects. The low hypoglycemic activity at a dose of 25 mg/kg body weight when given orally may suggest that the phytochemical content in this therapeutic dose was low and may have been degraded or inactivated by digestive enzymes or may have not attained saturable levels [15]. When given intraperitoneally, the hypoglycemic effects were significant within the first two hours (comparable to the reference drug) as opposed to the oral route where effects were gradual and reached maximum at the eighth hour. This could be attributed to active compounds reaching the systemic circulation faster [12] [16] and the fast pass metabolism realized in the intraperitoneal route [17].

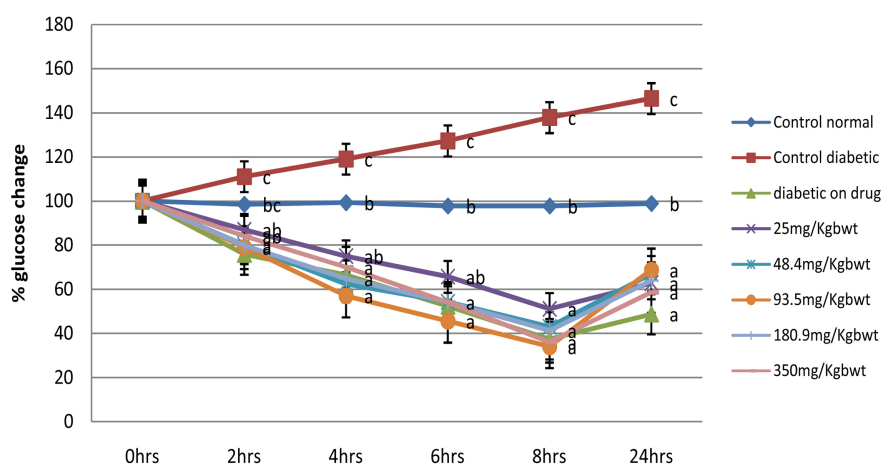
The effect in the extract was in a dose independent manner suggesting that the minimum dose was sufficient enough to effect maximum hypoglycemic activity. It could also suggest that the plant extract exert its effects in a saturable manner and this was in agreement with previous studies on aqueous plant extracts [18].

The studied plant extract contained phytochemicals alkaloids, saponins, flavonoids, phenols and tannins and a wide range of trace elements which in previous studies have demonstrated effects on the activity of pancreatic beta cells, increased inhibitory effect against insulinase enzyme, increased insulin sensitivity or the insulin-like activity of the plant extracts [19]. Flavonoids act as natural antioxidants and have an effect on many diseases [20]. They have anti-tumor, anti-inflammatory, anti-allergic, anti-thrombotic, anti-diabetic [21] and anti-atherosclerotic activities [22]. The alkaloid 1-ephedrine promotes the regeneration of pancreas islets following destruction of the beta cells, hence restores the secretion of insulin, and thus corrects hyperglycemia [23]. Phytonutrients are one of the components of food, though they are not synthesized in the body, they are essential for optimal health. Several essential metals are required for the proper functioning of many enzymes, transcriptional factors and proteins important in various biochemical pathways. For example Zn, Mg and Mn are cofactors of hundreds of enzymes, and Zn and Cr are co-factors in the synthesis and secretion of insulin from the pancreatic beta-cells [12] [24]. Similarly, Cr enhances the insulin receptor activity on target tissues, especially in muscle cells [25]. Copper (Cu) plays vital role in various metabolic processes. Ceruloplasmin ferroxidase activity and intracellular iron utilization is copper dependent. Superox-

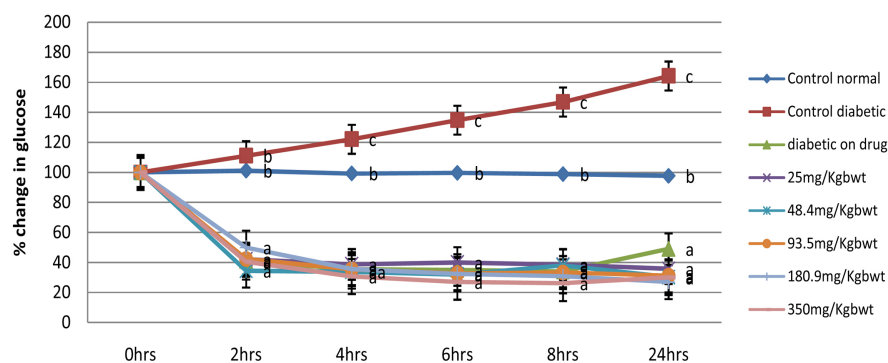
ide dismutase a powerful antioxidant is a Copper-Zinc dependent enzyme which protects cells against free radical injury just like the Manganese dependent superoxide dismutase [26] [33]. Severe copper deficiency in infants results in pathological bone fractures (cross-links collagen), cardiovascular disorders (cross-links soluble elastin and collagen) and emphysema-like lung condition which are associated with reduced activity of a copper dependent enzyme lysyl oxidase [27]. It can also cause neurological problems such as ataxia, seizures and episodic apnea which could be caused by lack of myelination leading to reduced nerve cell formation during embryonic development [28].

Iron (Fe) is an essential transition metal required for the synthesis of two important functional proteins such as hemoglobin and myoglobin, which are involved in the transport of molecular oxygen during respiration [29]. It is also required in the elastin production along with Zn and ascorbic acid and collagen synthesis. In blood stream small fraction of serum Fe is transported by glycoprotein transferrin into the cells. In the body tissues, ferritin stores free Fe, which is increased in newly diagnosed diabetic subjects [29]. [30] manifested higher level of ferritin in diabetics as compared to the non-diabetic subjects.

Chromium (Cr) regulates insulin and blood glucose levels by stimulating insulin signaling pathway and metabolism by up-regulating glucose transporter (GLUT4) translocation in muscle cells [31]. Nickel was found in detectable levels in *A. pluriseta* studies in rats and humans which indicated that Nickel deprivation depresses growth, reproductive performance, and plasma glucose and that it alters the distribution of other elements in the body, including calcium, iron, and zinc [32]. Calcium improves insulin sensitivity in some type 2 diabetic populations [33]. Potassium supplementation yields improved insulin sensitivity, responsiveness and secretion; insulin administration induces a loss of potassium; and a high potassium intake reduces the risk of heart disease, atherosclerosis, and cancer [26].



**Figure 1.** Mean percentage change in blood glucose after oral administration of aqueous extracts of *Chasmanthera dependens* at therapeutic doses in alloxan induced diabetic mice.



**Figure 2.** Mean percentage change in blood glucose after intraperitoneal administration of aqueous extracts of *Chasmanthera dependens* at therapeutic doses in alloxan induced diabetic mice.

## 5. Conclusion

The mean % change in glucose levels (Figure 1 & Figure 2) and the mean change in glucose values (Table 3) of the study findings reveal that *Chasmanthera dependens* has significant hypoglycemic activity in the lowest dose when administered either orally or intraperitoneally. The intraperitoneal route exhibit better efficacy than oral at all doses and the maximum effect is within the first eight hours. The plant extract also exhibited presence of phytochemicals and mineral elements essential in glucose metabolism.

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## Conflicts of Interest

The authors declare no conflict of interest in the manuscript.

## Author's Contribution

The first and fourth authors conducted the laboratory experiments while second and third authors reviewed the paper.

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