

# Antidiabetic and Safety of *Lantana rhodesiensis* in Alloxan Induced Diabetic Rats

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## Abstract

*Lantana rhodesiensis* Linn is used traditionally in the management of several diseases including diabetes mellitus; however, its efficacy and safety is not scientifically evaluated. The aim of this study was to determine *in vivo* hypoglycemic activity and safety of aqueous extracts of *L. rhodesiensis* in white male albino rats. Aqueous extracts were screened for their hypoglycemic activity in alloxan induced diabetic rats using the oral and intraperitoneal routes. The safety of these extracts was studied in rats orally or intraperitoneally administered with 1 g/kg body weight daily for 28 days by recording the changes in body and organ weight, hematological and biochemical parameters and histology. Mineral compositions of the extracts were estimated using total reflection X-Ray Fluorescence System (TRXF) while the types of phytochemicals present were assessed using standard procedures. Aqueous extracts orally and intraperitoneally administered at 50, 100 and 150 mg/kg body weight demonstrated hypoglycemic activity with the intraperitoneal route being more effective than the oral route. Oral and intraperitoneal dose of 1 g/kg body weight of the extracts significantly reduced the body weight gain, increased the testis and spleen, and decreased the lung weight; reduced the hemoglobin levels, red blood cell count, packed cell volume and increased the neutrophil count; decreased the activity of  $\gamma$ -glutamyltransferase and histologically mildly reduced lymphoid follicles. Orally, the same dose decreased the red blood cell count, packed cell volume, mean cell volume, monocyte and platelet count; increased the activity of lactate dehydrogenase, and creatine kinase. The extract contained phenols, tannins, flavonoids, alkaloids, sterols, terpenoids, cardiac glycosides, phylobatannins, resins, and bound anthrax quinones. Potassium, calcium, manganese, iron, lead and zinc levels in the extracts were below the recommended daily allowance. In conclusion, the observed hypoglycemic activity and slight toxicity could be associated with the phytonutrients present in this plant. This study recommends use of this plant as herbal medicine.

**Keywords:** *Lantana rhodesiensis*; Aqueous extracts; Hypoglycemic activity; Toxicity

## Introduction

Diabetes mellitus is a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1]. The level of hyperglycaemia associated diabetes increases the risk of microvascular damage (retinopathy, nephropathy and neuropathy). It is associated with reduced life expectancy, significant morbidity due to the related microvascular complications, increased risk of macrovascular complications (ischaemic heart disease, stroke and peripheral vascular disease), and diminished quality of life. In 2000, an estimated 171 million people in the world had diabetes and this is projected to increase to 366 million by 2030 [2].

Diabetes mellitus is classified into insulin dependent diabetes mellitus (IDDM) or Type 1, and Non-Insulin Dependent Diabetes Mellitus (NIDDM) or Type 2. The aetiological type named Type 1 encompasses cases due to pancreatic islet beta-cell destruction and are prone to ketoacidosis. Type 1 includes those cases attributable to an autoimmune process, as well as those with beta-cell destruction and who are prone to ketoacidosis for which neither an aetiology nor a pathogenesis is known (idiopathic). The type named Type 2 includes the common major form of diabetes which results from defect(s) in insulin secretion with a major contribution from insulin resistance [1].

Diagnosis of diabetes mellitus is based on measurement of glycosylated haemoglobin Alc (HbA1c) level (6.1-7.0), fasting (7.0

mmol/L or greater on two separate occasions) or random blood glucose level (11.0 mmol/L or greater) if classic symptoms of diabetes such as polyuria, polydipsia, weight loss, blurred vision, fatigue are present, or two hour oral glucose tolerance testing (7.8-10.0 mmol/L) [3].

In conventional medical practice, the present therapies of diabetes mellitus are reported to have side effects. Management of diabetes mellitus with insulin is associated with draw backs such as insulin resistance, anorexia nervosa, brain atrophy and fatty liver after chronic treatment. In addition, insulin dependent diabetes mellitus is managed using drugs that control hyperglycemia such as amylin analogues. Sulphonylureas, an oral antidiabetic drug, act by closure of ATP dependent channel. Metformin, a biguanide oral antidiabetic acts by limiting intestinal glucose absorption. Besides the side effects associated with the use of insulin, the side effects of most oral glucose-lowering drugs may include severe hypoglycemia at high doses, lactic

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acidosis, idiosyncratic liver cell injury, permanent neurological deficit, digestive discomfort, headache, dizziness and even death. It is therefore clear that because of the side effects associated with the present antidiabetic drugs, there is need to develop effective, safe and cheap drugs for diabetes management. Such effective, safe and cheap drugs could be obtained by using medicinal plants which have been used by humans to manage various diseases including diabetes since the dawn of civilization [4].

Plant based herbal medicines have been used to prevent or cure diseases including diabetes mellitus since the dawn of civilization because they are thought to be effective, safe and affordable to the common population in the underdeveloped and developing countries of the world. Among the plants used in the management of diabetes mellitus is the rare and endangered plant, *Lantana rhodesiensis* Linn. Its local name is mukenia (Kikuyu, Mbeere). *L. rhodesiensis* (*L. ukambensis* or *Lippia ukambensis*) of the family Verbenaceae, is a woody herb or small shrub under 2 m tall often multi-stemmed. The leaves of this plant are mostly opposite or in whorls of 3, ovate 1.8 cm long, the tip pointed, base narrowed, edge round-toothed, the surface sandpapery above but hairy below. The flowers are mauve-purple, the centre often yellow-white, each slightly 2-lipped, tubular, only 1.3 mm across, in a dense many flowered head, the short stalks of 2 cm, the whole shorter than the leaves, surrounded by large bracts at the base, as broad as the young flower head, over 1 cm, usually hairy and ribbed. The fruits are blue-purple berries, rounded, shiny, 2.4 mm across, containing one seed, covered at first by the membranous calyx. It is found in grassland and wooded grassland, open woodland, old cultivation, amongst granite rocks, bushland, secondary bushland, 100 - 2,100 m above sea level. In Africa, it is found in Tanzania, Kenya, Uganda, west of Cameroon, the Congo basin, Burundi, Rwanda, Sudan, Ethiopia, south of Malawi, Zambia, Zimbabwe and Mozambique. This plant grows naturally in the wild but it can be propagated by seed and cuttings. The stems are used for starting fire and as torches. The plant is suitable as an ornamental and hedge and provides forage for bees. Leaves are used as insect repellent. The ripe sweet berries collected during the rainy season are eaten by children fresh. The leaves are chewed, or pounded and soaked in warm water and the resulting liquid is drunk to treat coughs, fever and sores in the throat and on the tongue. Roots are boiled in water and drunk for rheumatism and generalized body pains (Sambaa) [5].

While aqueous leaf extracts of *L. wightiana* in the same family as *L. rhodesiensis* has been reported to demonstrate hypoglycemic activity in alloxan induced diabetic wistar rats 2-4 hours after a single oral administration of extracts at 200 and 400 mg/kg body weight [6], no other reported study has been performed on aqueous leaf extracts of *L. rhodesiensis* at lower and higher doses. In addition, because of its diversified pharmacological properties and uses, and the fact that the phytochemical composition and hence activity of *L. rhodesiensis* may vary from region to region and from season to season, route of drug administration and the extraction solvent, this study was performed to evaluate *in vivo* hypoglycemic activity and safety of orally and intraperitoneally administered aqueous leaf extracts of *L. rhodesiensis* in alloxan induced white male albino rats.

## Materials and Methods

### Study site

This study was undertaken at the Department of Biochemistry and Biotechnology, School of Pure and Applied Sciences, Kenyatta University in January 2008. Kenyatta University is 23 km from Nairobi off Thika Road.

### Collection of plant materials

Green leaves of *L. rhodesiensis* were collected in September 2007 were collected from Kiritiri Village, Kianjiru Location, Gachoka Division, Mbeere District, Embu County, Kenya. The plant was identified by a plant taxonomist, Mrs Peris Kamau at the East African Herbarium, Nairobi, Kenya and a voucher specimen deposited there for future reference.

### Preparation of the leaf and stem extracts

The plants parts collected were the stems and leaves. The stems and leaves were collected while green and dried under shade at room temperature for 28 days. The dried leaves were ground using an electric mill. The powdered leaves material were kept at room temperature away from direct sunlight in closed, dry plastic bags.

One hundred grams of leaves material was extracted in 1 liter of distilled water at 60°C in a metabolic shaker for 6 hours. After extraction, the extract was decanted into a clean dry conical flask and then filtered through folded cotton gauze into another clean dry conical flask. The filtrate was then freeze dried in 200 ml portions using a Modulyo Freeze Dryer (Edward England) for 48 hours. The freeze-dried sticky black paste was then weighed and stored in an airtight container at -20°C until used for bioassay.

### Experimental animals

The study used male Swiss White Albino rats (3-4 weeks old) that weighed 90-150 g with a mean weight of 120 g. These were bred in the Animal house at the Department of Biochemistry and Biotechnology of Kenyatta University. The rats were housed at a temperature of 25°C with 12 hours/12 hours darkness photoperiod and fed on rodent pellets and water *ad libitum*. The experimental protocols and procedures used in this study were approved by the Ethics Committee for the Care and Use of Laboratory Animals of Kenyatta University, Kenya.

### Induction of hyperglycemia

Hyperglycemia was induced experimentally by a single intraperitoneal administration of 186.9 mg/kg body weight of a freshly prepared 10% alloxan monohydrate (2,4,5,6 tetraoxypyrimidine; 5-6-dioxuracil) obtained from Sigma (Steinheim, Switzerland). Forty-eight hours after alloxan administration, blood glucose level was measured using a glucometer. Rats with blood glucose levels above 2000 mg/L were considered diabetic and used in this study. Prior to initiation of this experiment, the animals were fasted for 8-12 hours (Szkudelski, 2001) but allowed free access to water until the end of this experiment.

### Experimental design

For either intra-peritoneal or oral route of drug administration, the experimental rats were randomly divided into six groups of five animals each. Group I consisted of normal rats intra-peritoneally and orally administered with 0.1 ml physiological saline; Group II consisted of alloxan induced diabetic rats intra-peritoneally and orally administered with 0.1 ml physiological saline; Group IIIa consisted of alloxan induced diabetic rats intra-peritoneally administered with insulin (reference drug) (1 IU/kg body weight) in 0.1 ml physiological saline; Group IIIb consisted of alloxan induced diabetic rats orally administered with glibenclamide (reference drug) (3 mg/kg body weight) in 0.1 ml physiological saline; Group IV consisted of alloxan induced diabetic rats intraperitoneally and orally administered with 600 mg extract (50 mg/kg body weight) in 0.1 ml physiological saline; Group V consisted of alloxan induced diabetic rats intraperitoneally

and orally administered with 1200 mg extract (100 mg/kg body weight) in 0.1 ml physiological saline; and Group VI consisted of alloxan induced diabetic rats intraperitoneally and orally administered with 1800 mg extract (150 mg/kg body weight) in 0.1 ml physiological saline.

### Preparation of extracts for injection in rats

The appropriate doses of freeze-dried plant extracts were made by dissolving 600 mg (to make 50 mg/kg body weight), 1200 mg (to make 100 mg/kg body weight), and 1800 mg (to make 150 mg/kg body weight), in 10 ml physiological saline respectively. Insulin dose was prepared by dissolving 12 insulin units in 10 ml (1 IU/kg body weight) of physiological saline. 0.1 ml of the plant extract solution was administered either intra-peritoneally or orally to each experimental rat.

### Blood sampling and blood glucose, rate constant and half-life determination

Blood sampling was done by sterilizing the tail with 10% alcohol and then nipping the tail at the start of the experiment and repeated after 1, 2, 3, 4, 12 and 24 hours. The blood glucose levels were determined with a glucose analyser model (Hypogaurd, Woodbridge, England). The rate constant (k) was obtained by plotting log concentration of blood glucose for the first four hours against time in hours. This gave the pseudo-first order rate constant ( $k/2.303$ ) with a constant indicating the point where the straight line intersects the natural logarithm of glucose concentration axis (indicating the original glucose concentration before the drug administration) [7]. The half-life was calculated by substituting for the rate constant (k) in the formulae:  $t_{0.5} = 0.693/k$  whereby  $t_{0.5}$  is the time when the dosage has reduced the plasma sugar level by half [8]. The exponential decay equation was used to get the dosage that would be administered after a certain period [9].

### In vivo single dose toxicity test

The rats were randomly divided into four different groups of three rats each. Group I and II consisted of untreated control rats intraperitoneally and orally, respectively, administered daily for 28 days with 0.1 ml physiological saline. Group III and IV consisted of normal control rats intra-peritoneally and orally administered daily for 28 days with 120 mg (1 g/kg body weight) in 0.1 ml physiological saline. During this period, the rats were allowed free access to rat pellet and water and observed for any signs of general illness, change in behavior and mortality. At the end of 28 days, the rats were sacrificed.

### Determination of body and organ weight

The body weight of each rat was assessed once before commencement of dosing, once weekly during the dosing period, and prior to autopsy. All animals were euthanized for autopsy at the end of the experiment. The heart, liver, lungs, spleen, kidneys, brain, eyes and testis were carefully dissected out and weighed. These organ were then stored.

### Determination of hematological parameters

Blood parameters and indices were determined using standard protocols [10]. Red blood cells, white blood cells, hemoglobin, mean cell hemoglobin, mean cell hemoglobin concentration and mean cell volume were determined using the Coulter Counter (Beckman Coulter®, ThermoFisher, UK). Differential white blood cell count for neutrophils, lymphocytes, eosinophils, basophils and monocytes were determined from stained blood films using a hemocytometer [10]. Air-dried thin blood films stained with giemsa stain were examined microscopically using magnification 400 and 1000 for differential WBC

counts and cell morphologies, respectively. The other part was collected in plastic test tubes and allowed to stand for 3 hours to ensure complete clotting. The clotted blood samples were centrifuged at 3000 rpm for 10 min and clear serum samples were aspirated off and stored frozen at  $-20^{\circ}\text{C}$  until required for biochemical parameter analysis.

### Laboratory analysis of biochemical parameters

The biochemical parameters determined on the sera specimen using the Olympus 640 Chemistry Auto Analyser were Alanine Amino Transferase (AST), Aspartate Amino Transferase (ALT), Alkaline Phosphatase (ALP), Gamma Glutamyl Transferase (GGT), Lactate Dehydrogenase (LDH), Blood Urea Nitrogen (BUN), Creatinine (CREAT), Amylase (AMY) and Creatinine kinase (CK). 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.

### Quality Control (QC)

Precinorm U (normal upper) and precipath U (pathological upper) for all the parameters from Roche Diagnostics were the quality control materials used during the study period. Before use, a QC bottle was carefully opened and exactly 3 ml distilled water pipetted carefully into the bottle, closed, and carefully dissolved by gentle swirling within 30 minutes. This was then liquated into six cryovials and stored at  $-20^{\circ}\text{C}$ . Calibrator used the same types of tubes and racks as samples. A refrigerated rack position in the machine improved the stability of on-board controls. The system performed controls automatically according to the specifications in the test definition.

### Histopathology

Autopsy samples were collected and stored in 10% formalin. The tissues were processed using the standard protocols of histopathology. The heart, lungs, liver, kidney and testes were observed for any histopathological changes.

### Phytochemical screening

A phytochemical screening of total phenols, alkaloids, flavonoids, saponins, tannins, sterols, terpenoids, sterols, cardiac glycosides, phylobatannins, resins, free and bound anthraquinones present in *L. rhodesiensis* extracts was performed using standard methods [11,12]. For quantitative determination of phytochemicals, 2 g of the *L. rhodesiensis* extracts were defatted with 100 ml of diethyl ether using a Soxhlet apparatus for 2 hours. Total phenols were determined using the method described by [13], tannins were determined using the method described by [14], alkaloids were determined using the method described by [15], and flavonoids were determined using the method described by [16].

### Determination of mineral elements composition of leaf extracts of *L. rhodesiensis* using TXRF system

TXRF system was used to determine the content of potassium, calcium, iron, manganese, copper, zinc, bromine, rubidium and lead in the leaf extracts of *L. rhodesiensis*. Each freeze-dried sample were filtered and weighed. About 100 mg of homogenous sample

was pressed into 1 mm thick and 10 mm diameter and placed onto the sample tray [17]. The total reflection X-Ray Fluorescence System analysis consists of an x-ray spectrometer and a radioisotope excitation source. The radiation from the radioactive source,  $Cd^{109}$  (half-life,  $T_{1/2} = 453$  days and activity = 10 mCi) are incident on the sample that emits the characteristic X-rays. These X-rays are detected by Si (Li) detector (EG&G Ortec, 30 mm<sup>2</sup>\*10 mm sensitive volume, 25  $\mu$ m Be window) with an energy resolution of 200 eV at 5.9 keV Mn  $K_{\alpha}$  - line. The spectral data for analysis were collected using personal computer based Canberra S-100 Multi-Channel Analyser (MCA). The acquisition time applied in the TXRF measurement was 1000 seconds. For data analysis, the X-ray spectrum analysis and quantification was done using IAEA QXAS software [18] that is based on the Fundamental Parameters Method (FPM) [19,20]. By using this method, the composition of unknown sample is extrapolated by its fluorescence X-ray intensity of each element. The results are expressed in parts per million (ppm  $\approx$   $\mu$ g/g).

### Data management and statistical analysis

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 was done using ANOVA and post-ANOVA to compare the means of untreated normal control rats with diabetic rats treated with saline, diabetic rats treated with the conventional drug, diabetic rats treated with plant extracts at doses of 50, 100 and 150 mg per kg body weight.  $P < 0.05$  was considered statistically significant.

## Results

### Effects of intraperitoneal and oral administration of *L. rhodesiensis* leaf extracts at 50, 100 and 150 mg/kg body weight on blood glucose levels in alloxan induced diabetic rats

The aqueous leaf extract of *L. rhodesiensis* yielded 6.6% black granules. Intraperitoneal administration of aqueous leaf extracts of *L. rhodesiensis* decreased the blood glucose levels at all the three doses (50, 100 and 150 mg/kg body weight). This occurred in two phases, in the first two hours the extract caused a steep decline in blood glucose levels, followed by a steady decline in the third and fourth hour. After this, a gradual increase was recorded in the twelfth to the twenty fourth hours (Table 1). However, the sugar levels were not reduced in a dose dependent manner. In the first hour, the extracts lowered blood glucose levels to 71.8%, 64.2% and 55.3% for 50, 100 and 150 mg/kg body weight doses, respectively, compared to insulin treated diabetic rats whose blood sugar levels was lowered by 52.3% within the first hour (Figure 1). By the fourth hour, all the three doses (50, 100 and 150 mg/kg body weight) had lowered blood sugar levels by 31.4%, 35.9% and 31.1%, respectively, compared to insulin treated diabetic rats whose sugar levels was lowered by 21.5% within the same hour (Figure 1).

Oral administration of the aqueous leaf extracts of *L. rhodesiensis* also lowered blood glucose levels at all the three doses (50, 100 and 150 mg/kg body weight) from the first hour to the fourth hour and again in a dose-independent manner. In the first hour, the extracts lowered blood glucose levels to 52.5%, 54.3% and 57.9% for 50, 100 and 150 mg/kg body weight doses, respectively, compared to glibenclamide treated diabetic rats whose blood sugar levels was lowered by 49.3% within the same hour (Figure 2). By the fourth hour, all the three doses (50, 100 and 150 mg/kg body weight) had lowered blood sugar levels by 35.6%, 42.3% and 42.4%, respectively, compared to glibenclamide

treated diabetic rats whose sugar levels was lowered by 28.5% within the same hour. The reduction in blood glucose levels when compared to the negative control was statistically significant ( $p < 0.05$ ) (Figure 2).

Table 2 shows the pharmacokinetics of the hypoglycemic activity for the first four hours of the aqueous leaf extracts of *L. rhodesiensis*. Results indicate that the pseudo-first order rate constants for the three doses of the aqueous leaf extracts of *L. rhodesiensis* together with their accompanying half-lives are similar but lower than those of the intraperitoneal and oral conventional drugs. The rate constants for the aqueous leaf extracts for the three doses orally and intraperitoneally administered ranged from 0.1941 to 0.2731 per hour and the half-lives ranged from 3.57 to 2.54 hours, respectively. The rate constant for insulin was 0.3634 per hour and that of glibenclamide was 0.3132 per hour while the corresponding half-lives were 1.91 and 2.21 hours, respectively.

### Effects of intraperitoneal and oral administration of aqueous leaf extracts of *L. rhodesiensis* at 1 g/kg body weight daily for 28 days in rats on body weight gain, weekly body weight changes and percent relative organ weights

Tables 3 and 4a show the effect of intra-peritoneal and oral administration of aqueous leaf extracts of *L. rhodesiensis* at 1 g/kg body weight for 28 days in rats on body weight gain, weekly body weight changes and percent relative organ weights. Results show that oral administration of aqueous leaf extracts of *L. rhodesiensis* at a dose of 1 g/kg body weight in rats for 28 days significantly resulted in a decreased body weight gain and weekly body weight change and no effect on the percent relative organ weights except the spleen whose percent relative organ weight increased in the experimental rats compared to the control rats. In addition, intra-peritoneal administration of aqueous leaf extracts of *L. rhodesiensis* at the same dose in rats for 28 days significantly resulted in a decreased body weight gain and weekly body weight changes and increased the percent relative organ weights of the liver, testis, brain and spleen in the experimental rats compared to those of control rats.

### Effects of intraperitoneal and oral administration of a high dose of aqueous leaf extracts of *L. rhodesiensis* for 28 days in rats on some end point hematological parameters

Intraperitoneal administration of aqueous leaf extracts of *L. rhodesiensis* at 1 g/kg body weight to rats for 28 days significantly decreased the levels of hemoglobin, red blood cell count and packed cell volume; however, it had no significant effect on the other measured hematological parameters. This intraperitoneal dose increased the neutrophil count but had no significant effect on the white blood cells, lymphocytes, eosinophils, basophils and monocytes (Tables 4a and 5). Oral administration of aqueous leaf extracts of *L. rhodesiensis* at 1 g/kg body weight to rats for 28 days decreased the red blood cell count, packed cell volume, mean cell volume and platelet count but had no effect on all the other measured hematological parameters compared to those of the controls; this oral dose decreased the monocyte count but had no significant effect on the white blood cells, neutrophils, lymphocytes and eosinophils compared to those of the controls.

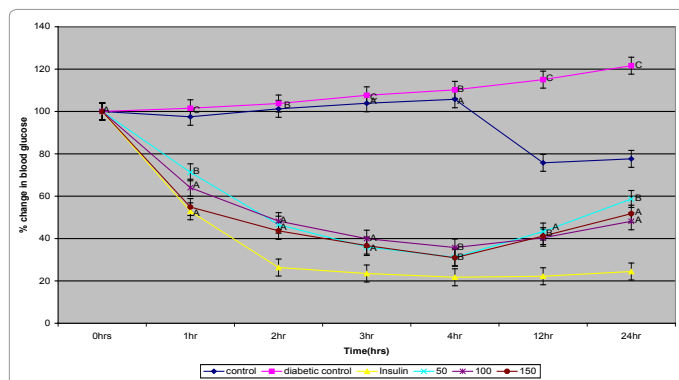
### Effects of intraperitoneal and oral administration of a high dose of aqueous leaf extracts of *L. rhodesiensis* for 28 days in rats on some end point biochemical parameters

Oral administration of aqueous leaf extracts of *L. rhodesiensis* at 1 g/kg body weight to rats for 28 days significantly decreased the activities of Lactate Dehydrogenase (LDH) and creatine kinase (CK);

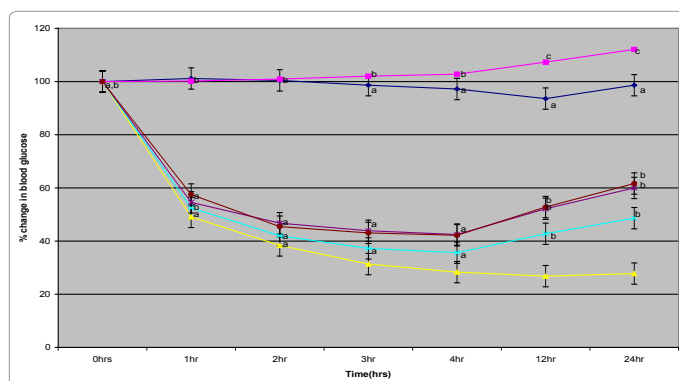
Treatment	Route	Glucose levels at varying times (mg/dL)						
		0 hr	1 hr	2 hr	3 hr	4 hr	12 hr	24 hr
Group I	IP	66.75 ± 10.78	65.00 ± 9.45	67.50 ± 7.76	69.25 ± 4.99 <sup>A</sup>	70.50 ± 8.81 <sup>A</sup>	50.50 ± 2.65	51.75 ± 2.22
	Oral	69.50 ± 1.92	70.25 ± 3.30	69.75 ± 2.22	68.50 ± 2.65 <sup>a</sup>	67.50 ± 2.38 <sup>a</sup>	65.00 ± 6.48 <sup>a</sup>	68.50 ± 1.29 <sup>a</sup>
Group II	IP	212.50 ± 10.83 <sup>A</sup>	216.00 ± 11.14 <sup>C</sup>	220.75 ± 9.74 <sup>B</sup>	229.00 ± 9.49 <sup>C</sup>	234.50 ± 8.39 <sup>B</sup>	244.75 ± 7.27 <sup>C</sup>	258.75 ± 7.59 <sup>C</sup>
	Oral	203.25 ± 8.96 <sup>b</sup>	204.50 ± 8.89 <sup>b</sup>	206.00 ± 8.83 <sup>b</sup>	208.25 ± 9.54 <sup>b</sup>	209.75 ± 9.47 <sup>b</sup>	219.00 ± 10.10 <sup>c</sup>	228.75 ± 7.93 <sup>c</sup>
Group III	IP	215.00 ± 11.40 <sup>A</sup>	112.50 ± 6.86 <sup>A</sup>	56.00 ± 10.30	50.00 ± 9.13	46.25 ± 4.50	47.25 ± 1.71	52.00 ± 0.82
Group IV	Oral	181.00 ± 12.57 <sup>a</sup>	89.25 ± 6.02 <sup>a</sup>	69.75 ± 2.23	57.00 ± 3.16	51.50 ± 1.29	48.75 ± 0.96	50.50 ± 1.29
Extract								
Group V	IP	230.75 ± 23.47 <sup>A</sup>	165.75 ± 45.33 <sup>B</sup>	108.25 ± 19.60 <sup>A</sup>	83.75 ± 5.25 <sup>A</sup>	72.50 ± 5.45 <sup>B</sup>	100.75 ± 4.86 <sup>B</sup>	136.50 ± 21.30 <sup>B</sup>
	Oral	200.00 ± 18.96 <sup>b</sup>	105.00 ± 7.62 <sup>b</sup>	84.00 ± 4.08 <sup>a</sup>	74.50 ± 5.51 <sup>a</sup>	71.25 ± 5.32 <sup>a</sup>	85.50 ± 11.73 <sup>b</sup>	97.25 ± 12.45 <sup>b</sup>
Group VI	IP	221.50 ± 13.48 <sup>A</sup>	142.25 ± 33.63 <sup>A</sup>	107.25 ± 9.88 <sup>A</sup>	88.75 ± 4.79 <sup>B</sup>	79.50 ± 2.65 <sup>A</sup>	89.75 ± 2.22 <sup>A</sup>	107.00 ± 6.68 <sup>A</sup>
	Oral	176.25 ± 16.88 <sup>a</sup>	95.75 ± 3.86 <sup>a</sup>	82.00 ± 2.94 <sup>a</sup>	77.00 ± 3.92 <sup>a</sup>	74.50 ± 5.69 <sup>a</sup>	91.50 ± 2.65 <sup>b</sup>	105.25 ± 3.30 <sup>b</sup>
Group VII	IP	220.75 ± 24.85 <sup>A</sup>	122.00 ± 17.34 <sup>A</sup>	97.00 ± 8.29 <sup>A</sup>	81.50 ± 7.85 <sup>A</sup>	68.75 ± 3.60 <sup>A</sup>	91.50 ± 6.66 <sup>A</sup>	115.00 ± 2.58 <sup>A</sup>
	Oral	168.50 ± 14.06 <sup>a</sup>	97.50 ± 5.80	77.00 ± 4.69 <sup>a</sup>	73.00 ± 3.65 <sup>a</sup>	71.50 ± 3.70 <sup>a</sup>	89.50 ± 8.10 <sup>b</sup>	104.50 ± 9.32 <sup>b</sup>

Results are expressed as Means ± SEM for four animals per group. Means within respective columns followed by <sup>a</sup> are significantly different at  $p \leq 0.05$  (Student *t* test); Means for intra-peritoneally (Ip) administered drugs followed by similar upper case letters are not significantly different at  $p \leq 0.05$  by ANOVA and Bonferroni-Holm test; Means for orally (oral) administered drugs followed by similar lower case letters are not significantly different at  $p \leq 0.05$  by ANOVA and Bonferroni-Holm test.

**Table 1:** Effects of three therapeutic doses of aqueous leaf extracts of *L. rhodesiensis* at different times on blood glucose levels in alloxan induced diabetic rats



**Figure 1:** Mean percentage change in blood glucose levels of *L. rhodesiensis* administered intraperitoneally in alloxan induced diabetic rats. Values are expressed as % means ± SEM for four animals for each time point. <sup>a</sup> $p < 0.05$  when compared to normal control; <sup>a</sup> $p < 0.05$  when compared to diabetic control and <sup>b</sup> $p < 0.05$  when compared to diabetic control.



**Figure 2:** Mean percentage change in blood glucose levels of *L. rhodesiensis* administered orally in alloxan induced diabetic rats. Values are expressed as % means ± SEM for four animals for each time point. <sup>a</sup> $p < 0.05$  when compared to normal control; <sup>a</sup> $p < 0.05$  when compared to diabetic control and <sup>b</sup> $p < 0.05$  when compared to diabetic control.

however, it had no significant effect on the activities of Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP),  $\gamma$ -glutamyltransferase (GGT) and Amylase (AMY)

(Table 6). Intraperitoneal administration of aqueous leaf extracts of *L. rhodesiensis* at 1 g/kg body weight to rats for 28 days significantly decreased the activity of  $\gamma$ -glutamyltransferase (GGT), but had no significant effect on the activities of Alanine Aminotransferase (ALT), Lactate Dehydrogenase (LDH), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Alkaline Phosphatase (ALP) and Amylase (AMY) (Table 6).

### Histopathology

Oral administration of 1 g/kg body weight of aqueous extracts of *L. rhodesiensis* did not show any pathology to the liver, kidney, heart, lungs, testis, brain, and eyes. However, intraperitoneal administration of the same resulted in histological changes in the spleen. The spleen sections of rats intraperitoneally administered with aqueous leaf extracts of *C. pareira*, showed reduction in cell density of lymphoid follicles (Plate 1).

### Phytochemical screening of aqueous leaf extracts of *L. rhodesiensis*

Results show that aqueous extracts of *L. rhodesiensis* contained detectable levels of phenols, alkaloids, flavonoids, tannins, terpenoids, sterols, cardiac glycosides, phylobatannins, resins, and bound anthraquinones. The aqueous leaf extracts of *L. rhodesiensis* lacked detectable levels of saponins, reducing sugars and free anthraquinones. Quantitatively, the levels of phenols and tannins were  $685.25 \pm 30.77$  and  $323.61 \pm 61.54$  mg/g gallic acid equivalent, respectively while flavonoids and alkaloids were  $187.33 \pm 54.97$  and  $32.67 \pm 10.07$  mg/g, respectively (Table 7).

### Mineral element composition of the aqueous leaf extracts of *L. rhodesiensis* ( $\mu\text{g/g}$ ) and the quantity of each mineral in 1 g plant extracts per kg body weight orally and intra-peritoneally administered to each rat per day ( $\mu\text{g/day}$ )

Table 8 shows the minerals composition of the aqueous leaf extracts of *L. rhodesiensis* ( $\mu\text{g/g}$ ) and the quantity of each mineral administered in 1 g plant extracts per kg body weight to each rat per day ( $\mu\text{g/day}$ ). Results show that the aqueous leaf extracts of *C. pareira* contained detectable levels of Potassium (K), Calcium (Ca), Manganese (Mn), Iron (Fe), Zinc (Zn), Rubidium (Rb), Bromine (Br), and Lead (Pb), and undetectable quantities of Copper (Cu). In addition, these results indicate that the extracts provided potassium, calcium, manganese, iron and zinc at levels below the recommended daily allowance.

Drug (dose)	Route	Rate constant (hour <sup>-1</sup> )	Half-life (hours)
Insulin ((1 IU/kg body weight)	IP	<b>0.3634</b>	<b>1.91</b>
Glibenclamide (3 mg/kg body weight)	Oral	0.3132	2.21
Extract (mg/kg body weight)			
50	IP	<b>0.2731</b> ↑	<b>2.54</b> ↓
	Oral	0.2049	2.88
100	IP	<b>0.2259</b> ↑	<b>3.07</b> ↓
	Oral	0.1941	3.57
150	IP	<b>0.2393</b> ↑	<b>2.90</b> ↓
	Oral	0.2004	3.46

Results are expressed as Means of four rats for each time point

**Table 2:** Pharmacokinetics of the hypo-glycemic activity for the first four hours of the three doses of the aqueous leaf extracts of *L. rhodesiensis*

Treatment	Weekly Weight of Rats in g					ΔWeight/Week
	0	1	2	3	4	
Control (Oral)	128.7 ± 3.2	142.3 ± 2.1	159.0 ± 6.0	184.0 ± 6.0	206.7 ± 16.0	19.50 ± 3.18
<i>L. rhodesiensis</i>	146.0 ± 20.0*	151.0 ± 19.0*	157.0 ± 18.3	163.3 ± 18.5	170.3 ± 18.4*	6.08 ± 0.38*
Control (IP)	128.3 ± 2.1	147.0 ± 3.6	180.0 ± 26.5	200.0 ± 26.5	231.7 ± 20.2	25.83 ± 4.53
<i>L. rhodesiensis</i>	120.7 ± 4.5*	138.0 ± 4.6	152.3 ± 2.5*	156.7 ± 2.9*	163.0 ± 2.7*	10.58 ± 0.47*

Results are expressed as Mean ± Standard Deviation (SD) for three 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:** Effects of oral and intra-peritoneal administration of aqueous extracts of *L. rhodesiensis* at 1 g/kg body weight daily in rats for 28 days on body weight

Treatment	Liver	Kidney	Lungs	Heart	Testis	Brain	Spleen	Eyes
Control (Oral)	4.89 ± 0.36	0.78 ± 0.07	1.11 ± 0.09	0.41 ± 0.08	1.46 ± 0.12	1.04 ± 0.06	0.51 ± 0.05	0.18 ± 0.03
<i>L. rhodesiensis</i>	4.98 ± 0.30	0.88 ± 0.20	1.16 ± 0.05	0.49 ± 0.10	1.51 ± 0.09	1.06 ± 0.07	<b>0.66 ± 0.06*</b>	0.22 ± 0.04
Control (IP)	4.34 ± 0.49	0.70 ± 0.08	0.90 ± 0.02	0.43 ± 0.09	1.46 ± 0.14	0.75 ± 0.07	0.52 ± 0.06	0.11 ± 0.03
<i>L. rhodesiensis</i>	5.97 ± 0.21*	0.88 ± 0.10	1.00 ± 0.09	0.42 ± 0.03	2.50 ± 0.05*	1.00 ± 0.03*	1.25 ± 0.13*	0.14 ± 0.02

Results expressed as Mean ± Standard deviation (SD) for three animals for each parameter. Body weight gain and organ weight are measured in g. The high dose of *L. rhodesiensis* extracts is 1g/kg body weight. \*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 4a:** Effects of oral and intra-peritoneal administration of a high dose of aqueous leaf extracts of *L. rhodesiensis* in rats for 28 days on body weight gain and percent relative organ weight

Treatment	Hematological Parameters						
	Hb (g/dL)	RBC(x10 <sup>12</sup> /L)	PCV (%)	MCH (pg)	MCHC(g/dL)	MCV (fL)	PLT (x10 <sup>3</sup> /μL)
Control (Oral)	13.40 ± 0.44	6.83 ± 0.12	39.40 ± 0.75	20.53 ± 0.80	41.06 ± 0.35	69.47 ± 0.85	602.33 ± 19.01
<i>L. rhodesiensis</i>	13.37 ± 0.31	6.60 ± 0.36*	32.23 ± 1.72*	18.87 ± 0.31	39.33 ± 2.28	60.93 ± 0.71*	414.33 ± 51.29*
Control (IP)	13.00 ± 0.53	6.29 ± 0.48	40.03 ± 1.72	20.73 ± 0.90	32.47 ± 0.06	63.78 ± 2.57	552.67 ± 50.25
<i>L. rhodesiensis</i>	10.13 ± 0.55*	5.01 ± 0.23*	31.57 ± 1.97*	20.20 ± 0.95	32.13 ± 0.99	63.00 ± 1.90	367.67 ± 67.51

Results are expressed as Mean ± Standard Deviation (SD) for three animals in each treatment; the high dose of *L. rhodesiensis* extracts is 1g/kg body weight. \*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 4b:** Effects of intra-peritoneal and oral administration of a high dose of aqueous leaf extract of *L. rhodesiensis* in rats for 28 days on hematological parameters

Treatment	WBC(x10 <sup>9</sup> /L)	NEU (x10 <sup>9</sup> /L)	LYM (x10 <sup>9</sup> /L)	EOS (x10 <sup>9</sup> /L)	BASO(x10 <sup>9</sup> /L)	MONO(x10 <sup>9</sup> /L)
Control (Oral)	11.31 ± 1.23	6.48 ± 0.73	3.30 ± 0.46	0.78 ± 0.07		0.75 ± 0.07
<i>L. rhodesiensis</i>	9.73 ± 0.90	5.68 ± 0.79	3.11 ± 0.22	0.56 ± 0.17		0.39 ± 0.08*
Control (Ip)	10.53 ± 1.55	5.64 ± 0.69	3.20 ± 0.62	0.81 ± 0.19	0.18 ± 0.08	0.71 ± 0.16
<i>L. rhodesiensis</i>	11.10 ± 1.61	7.30 ± 0.64*	2.43 ± 0.56	0.78 ± 0.12	0.11 ± 0.02	0.49 ± 0.24

Results are expressed as Mean ± Standard deviation (SD) for three animals in each treatment; the high dose of *L. rhodesiensis* extracts is 1g/kg body weight. NEU represents Neutrophils, LYM represents Lymphocytes, EOS represents Eosinophils, BASO represents Basophils, and MONO represents Monocytes. \*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:** Effects of intra-peritoneal and oral administration of a high dose of aqueous leaf extracts of *L. rhodesiensis* in rats for 28 days on white blood cells (WBC) and the absolute differential white blood cell count

## Discussion

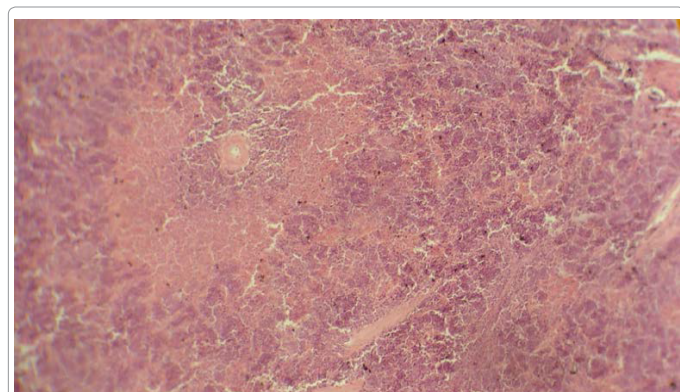
The alloxan-induced diabetic rats had a three to four fold increase in blood glucose (150 mg/dL to 250 mg/dL) relative to the normal control rats. Alloxan destroys and reduces the β-cells via formation of reactive oxygen species like nitric oxide [21]. The aqueous leaf extract of *L. rhodesiensis* showed blood glucose lowering effect in a dose independent manner when administered intra-peritoneally and orally

indicating that they contained hypoglycemic constituents. However, the intra-peritoneal route had a greater glucose reduction rate and a shorter half-life than the oral route. The greater glucose reduction rate and a short half-life of the intra-peritoneal route could be associated with the immediate higher bioavailability of active constituents to the systemic circulation while in the oral route the active constituents required initial transportation across the intact intestinal wall [22].

Treatment	Enzyme activities						
	ALT (U/L)	AST (U/L)	ALP (U/L)	GGT (U/L)	LDH (U/L)	CK (U/L)	AMY(U/L)
Control (Oral)	26.30 ± 8.00	32.30 ± 8.50	240.00 ± 52.80	15.00 ± 3.60	149.00 ± 17.10	116.0 ± 34.9	99.0 ± 11.4
<i>L. rhodesiensis</i>	43.0 ± 7.00	48.30 ± 13.60	190.00 ± 24.60	15.70 ± 3.50	208.8 ± 7.2*	156.7 ± 25.6*	98.7 ± 4.5
Control (IP)	46.7 ± 11.0	30.3 ± 6.0	218.7 ± 49.2	38.3 ± 3.2	227.3 ± 16.8	182.7 ± 9.0	78.3 ± 11.7
<i>L. rhodesiensis</i>	40.0 ± 9.5	44.7 ± 4.6	239.3 ± 48.4	25.7 ± 1.5*	216.7 ± 12.2	171.0 ± 27.6	79.0 ± 10.4

Results are expressed as Mean ± Standard deviation (SD) for three animals in each treatment; the high dose of *L. rhodesiensis* extracts is 1g/kg body weight. \*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:** Effects of intraperitoneal and oral administration of a high dose of aqueous leaf extracts of *L. rhodesiensis* in rats for 28 days on biochemical parameters



**Plate 1:** Histological section of a spleen of a rat treated intraperitoneally with an aqueous leaf extract of *L. rhodesiensis* (1g/kg body weight/day) for 28 days. There is mild reduction of lymphoid follicles (arrow). Magnification × 400

The lowering effect of blood sugar levels by *L. rhodesiensis* in the same manner regardless of the dosage might suggest that the extract may have been absorbed in the cell system through active transport where a particular concentration saturation of the extract occurred resulting to the rest of extract being excreted. The glucose lowering effect of aqueous leaf and ethanoic fruit extracts of *L. wightiana* and *L. camara* Linn respectively has previously been demonstrated by [23,24].

The blood glucose lowering effect of this plant extracts may be attributed to the presence of phenols, flavonoids, tannins, alkaloids, terpenoids, sterols and cardiac glycosides that have been associated with hypoglycemic activity [25]. The presence of flavonoids, sterols and saponins has previously been reported in ethanoic fruit extracts of *L. camara* Linn which demonstrated hypoglycemic activity in streptozotocin induced diabetic male wistar rats [24]. As reported by [26], flavonoids like myricetin, a polyhydroxylated flavonol has insulinomimetic properties and stimulate lipogenesis and glucose transport in the adipocytes hence lowering blood sugar [25]. Similar studies done on *Pterocarpus marsupium* found epicatechin and catechin flavonoids having anti-diabetic properties [27].

The alkaloids present in the aqueous leaf extract of this plant have also been reported to have antihyperglycemic activity. 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 [25]. The aqueous leaf extracts of the same plant contained tannins that are known to have hypoglycemic activity. The tannin epigallo-catechin-3-gallate exhibits anti-diabetic activity as demonstrated by [28].

The aqueous leaf extracts of *L. rhodesiensis* contains terpenoids which are heart-friendly phytochemical constituents; terpenoids are very popular among patients with high blood pressure and diabetes because they help to reduce diastolic blood pressure and lower the sugar level in blood [29]. Terpenoids also strengthen the skin, increase

the concentration of antioxidants in wounds, and restore inflamed tissues by increasing blood supply [29]. Terpenoids also improve lung function [29] and therefore make *L. rhodesiensis* a potential drug for use in the management of painful respiratory problems such as dyspnoea and oligopnoea. Due to the presence of terpenoids, the leaves and seeds of *E. officinalis* are used in the treatment of diabetes [30]. *L. rhodesiensis* extracts contain anthraquinones which have previously been reported to lower blood glucose [31] reported that *Polygonum multiflorum* extracts containing anthraquinones are used in the treatment of peripheral neuropathy, a complication associated with diabetes mellitus.

The steroids and phylobatannins present in this plant make it a good source of steroidal compounds which are potent precursors for the synthesis of sex hormones [32,33]. The presence of phenolic compounds in the leaf extracts of *L. rhodesiensis* indicates its antimicrobial properties against pathogenic bacteria [34,35]. Cardiac glycosides are helpful in overcoming various human diseases. Cardiac glycosides present in *L. rhodesiensis* have been shown to aid in treatment of congestive heart failure and cardiac arrhythmia. This is could be another reason why this plant is widely used in traditional medicine. Cardiac glycosides inhibit the Na<sup>+</sup>-K<sup>+</sup>-pump. The increase in the level of sodium ions in the myocytes, leads to a rise in the level of calcium ions. This inhibition increases the amount of Ca<sup>2+</sup> ions used in heart muscle contraction resulting in the improvement of cardiac output and reduction in the distention of the heart. The glycosides also possess strong antibacterial properties. Antibiotics such as streptomycin, neomycin, kanamycin, paromomycin, gentimycin and tobromycin are glycosides [36,37].

The hypoglycemic effect of this plant extract could also be attributed to the presence of iron, manganese and zinc [38]. Iron influences glucose metabolism and reciprocally, iron influences insulin action. Iron interferes with insulin inhibition of glucose production by the liver [39]. Calcium is required for normal growth and development of the skeleton. Extraskelatal calcium plays a role in mediating vascular contraction and vasodilation, muscular contraction, nerve transmission, glandular secretion and as a second messenger. Calcium levels below the recommended daily allowance as observed in this study reduces bone mass and cause osteoporosis; it may cause hypertension including pre-clampsia and colon cancer and also play a role in body weight regulation. Hyperkalemia and renal insufficiency with or without alkalosis occurs in potassium excesses and provokes a reduction in the absorption of iron, zinc, magnesium and phosphorus. Calcium inhibits the absorption of iron in a dose-dependent and dose saturable fashion [40]. Potassium overdose causes hyperkalemia which can lead to cardiac arrest. Other causes of hyperkalemia are either a shift of potassium from cells to the Extra Cellular Fluid (ECF) or excessive potassium retention caused by major trauma and infection, metabolic acidosis, Addison's disease (aldosterone insufficiency) or chronic renal failure. As expected from the close metabolic interactions among the major electrolytes, potassium and sodium dietary interactions are

Phenols (mg/g GAE)	Tannins (mg/g GAE)	Flavonoids (mg/g)	Alkaloid (mg/g)	Saponins (mg/g)
685.3 ± 30.8	323.6 ± 61.5	187.3 ± 55.0	32.7 ± 10.1	-
Results are expressed as Mean ± standard deviation (SD) of three determinations per extract				

**Table 7:** Quantitative phytochemical composition of the six aqueous plants extracts

Mineral	Mineral levels in the leaf extracts (µg/g)	Daily mineral administered (µg/day)	RDA for rats/day (µg/day)
K	60400 ± 4400	7.248 ± 0.528 x 10 <sup>3</sup>	2 x 10 <sup>6</sup>
Ca	13900 ± 1400	1.668 ± 0.168 x 10 <sup>3</sup>	1 x 10 <sup>6</sup>
Mn	153.0 ± 22.0	18.38 ± 2.64	2.3 x 10 <sup>3</sup>
Fe	123.0 ± 14.0	14.76 ± 1.68	8 x 10 <sup>3</sup>
Cu	< 20	<2.4	1.5 x 10 <sup>3</sup>
Zn	45.3 ± 3.7	5.436 ± 0.444	11 x 10 <sup>3</sup>
Pb	8.58 ± 1.3	1.0296 ± 0.156	-
Br	23.3 ± 2.1	2.796 ± 0.252	0.08
Rb	38.5 ± 3.7	4.62 ± 0.444	0.005

Results are expressed as Mean ± standard deviation (SD) for three determinations. < 1 is below the limit of detection of TXRF.

**Table 8:** Minerals composition of the aqueous leaf extracts of *L. rhodesiensis* (µg/g) and the quantity of each mineral in 1g plant extracts per kg body weight orally and intra-peritoneally administered to each rat per day (µg/day)

important in determining the risk of coronary heart disease and stroke. Potassium has a positive effect on calcium balance by regulating the acid-base balance and ameliorating any effects of sodium on calcium depletion [40]. Overdose of manganese causes “manganic madness,” manifested by psychosis, hallucinations, and extrapyramidal damage with features of Parkinsonism [40].

Iron deficiency increases manganese absorption, and high amounts of dietary iron inhibit manganese absorption by competing for similar binding and absorption sites between nonheme iron and manganese [40]. Manganese is an activator and constituent of several enzymes like kinases and enzymes of oxidative phosphorylation [41]. Zinc also influences glyceraldehyde-3-phosphate dehydrogenase, the enzyme involved in glycolysis [42].

Because the toxicity of a drug to the host cells could render it unsuitable for therapeutic purposes, the toxicity of high dose of this plant extract was assessed in rats. The reduced weight gain in rats administered with 1 g/kg body weight for 28 days relative to the control rats suggests that this extract contained phytochemical constituents which promoted degradation of proteins from skeletal muscles and hence retarded growth [24]. Such phytochemicals may include alkaloids, tannins, terpenoids, saponins, flavonoids, coumarins, anthocyanins and sugars which have previously been reported to be toxic. Saponins hemolyse red blood cells and cause cell death of many tissues [43,44]. 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 [44]. However, saponins were absent in *L. rhodesiensis* aqueous extracts. Alkaloids have been reported to cause liver megalocytosis, proliferation of biliary tract epithelium, liver cirrhosis and nodular hyperplasia [45]. However, these changes were not observed in this study. Some of the toxic alkaloids found in plants include confine, solanine, methyllycaconitine, nudicauline, and geyerline [46], 2-pentylpiperidine [47], and pyrrolizidine alkaloids [48]. Terpenoids have been reported to increase membrane permeability to divalent and monovalent ions [45]. Long-term or repeated exposure of phenols to animals has been reported to lead to histo-pathological changes to liver and kidney [49].

Tannins reduce feed intake by decreasing palatability and by reducing feed digestion. Palatability is reduced because tannins are astringent. Astringency is the sensation caused by the formation of complexes between tannins and salivary glycoproteins. Low palatability

depresses feed intake. Tannins are divided into two: hydrolysable and condensed tannins. Hydrolysable tannins are converted by microbial metabolism and gastric digestion into absorbable low molecular weight metabolites such as tannic acid which are toxic. The major lesions associated with hydrolysable tannins poisoning are hemorrhagic gastroenteritis which decreases absorption of nutrients, necrosis of the liver, and kidney damage with proximal tubular necrosis.

Protanthocyanidins (PAs) (condensed tannins) retard growth by inhibiting feed intake and digestibility [50]. Proanthocyanidins (PAs) which are not absorbed by the digestive tract, damage the mucosa of the gastrointestinal tract, decreasing the absorption of nutrients such as proteins and carbohydrates and essential amino acids such as methionine and lysine. They also increase excretion of proteins and essential amino acids and alter the excretion of certain cations. Mineral element overdose may also cause toxicity but this was not the case with the measured minerals since their levels were below the recommended daily allowance except for bromine and rubidium which were beyond the recommended daily allowance.

The decreased lung weight of rats intraperitoneally administered with 1 g of aqueous extracts of *L. rhodesiensis* per kg body weight daily for 28 days correlated with the reduced growth rate. The increased testis weight of rats intraperitoneally administered with 1 g of aqueous extracts of *L. rhodesiensis* per kg body weight daily for 28 days could be attributed to an induction of a higher metabolic activity by some phytochemical constituents in this organ.

A daily intraperitoneal administration of 1 g of the aqueous extracts of *L. rhodesiensis* per kg body weight to rats for 28 days induced reduction of red blood cell count, hemoglobin levels, and packed cell volume leading to normocytic anemia. The significantly raised neutrophils suggest a direct effect on the myeloid stem cell component in the bone marrow by the toxic phytoconstituents in the extracts or a reaction to bacterial infection [51]. Increased levels of neutrophils imply an increased ability to protect the body against bacteria. A daily oral administration of 1 g of the aqueous extracts of *L. rhodesiensis* per kg body weight to rats for 28 days induced a reduction in red blood cell count, packed cell and mean cell volume leading to microcytic anemia. The significantly decreased monocyte suggest either a direct effect on the myeloid stem cell component in the bone marrow or the lymphoid stem cell component in the spleen caused by the toxic phytoconstituents in the extracts; spleen injury was histologically reflected in the form of lymphoid follicle depopulation.



Reduced levels of monocytes imply impaired phagocytosis of foreign matter. The significantly decreased platelet count could either be associated with direct toxicity to the thrombocytes or depression of thrombocyte production by phytoconstituents in the extracts such as steroids or lithium [51]. Normocytic anemia may be caused by a toxic effect on the erythropoietic precursor cells in the bone marrow by the phytochemical constituents in the extracts. The toxic constituents include alkaloids, flavonoids, and tannins present in this extract which have been reported to reduce erythron parameters [52]. However, these erythrocytic variations were mild and did not lead to overt organ injury after oral administration of 1 g of the extract of *L. rhodesiense* per kg body weight to rats for 28 days. This observation contrasts with the alterations in the activities of Lactate Dehydrogenase and creatine kinase. Microcytic anemia may be due to induction of iron deficiency due to malabsorption of nutrients brought about by high tannin levels present in these extracts. A reduction in the red blood cell count as observed after daily intraperitoneal and oral administration of 1 g of the aqueous extract of *L. rhodesiense* per kg body weight to rats for 28 days causes tissue hypoxia.

Tissue hypoxia causes most tissues to initially enlarge and as the swollen cells continue rupturing, the organ size reduces (organ atrophy) [53]. During tissue hypoxia, cells which rely only on glycolysis for ATP production rapidly deplete the store of phosphocreatine (a source of rapid ATP production) and glycogen. As the rate of ATP production decreases below the level required by membrane ion pumps for the maintenance of proper intracellular ionic concentrations, the osmotic balance of the cell is disrupted so that the cell and its membrane enveloped organelles swell. The overstretched membrane becomes permeable thereby leaking their enclosed contents. The decreased intracellular pH that accompanies anaerobic glycolysis because of lactic acid production permits the released lysosomal enzymes which are only active at acidic pH to degrade the cell contents. The reduced metabolic activity results in irreversible cell damage [53]. Injury of organs resulting from tissue hypoxia may account for the decreased lung and increased testis weight. While Injury of organs resulting from tissue hypoxia was not histologically demonstrated in this study, it is possible that subcellular damage to organs may account for the altered serum activities of Lactate Dehydrogenase (liver, kidney, heart), creatine kinase (heart, skeletal muscle) and  $\gamma$ -glutamyltransferase (liver) in rats both intraperitoneally and orally administered daily with 1 g of aqueous extracts of *L. rhodesiense* per kg body weight for 28 days [54-58].

## Conclusion

In conclusion, *L. rhodesiense* used traditionally in the practice of herbal medicine has demonstrated antidiabetic activity when therapeutic doses were administered intra-peritoneally and orally. The intra-peritoneal route was more effective than the oral route as assessed from the rate of glucose disappearance and its half-life. A very high dose of *L. rhodesiense* (1 g/kg body weight) that is far from the therapeutic dose is likely to produce toxicological effects as assessed by reduced body weight gain, changes in lung and testis weight, alterations in hemoglobin levels, packed cell and mean cell volume, red blood cell, platelet, neutrophil and monocyte count and alterations in the activity of lactate dehydrogenase, creatine kinase and  $\gamma$ -glutamyltransferase and histopathological changes on the spleen. The antidiabetic activity and the toxicity of a high dose of this plant may be accounted for by the phytonutrients present in this plant.

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