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In Vivo Antidiabetic Activity and Safety In Rats of *Cissampelos pareira* Traditionally Used In The Management of Diabetes Mellitus In Embu County, Kenya

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Abstract

Cissampelos pareira Linn has been used traditionally in the management of several diseases including diabetes mellitus but its efficacy and safety after long term use is not scientifically evaluated. The aim of this study was to determine in vivo hypoglycemic activity and safety of aqueous leaf extracts of C. pareira in white male albino rats. The 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 composition of the extracts were estimated using total reflection X-ray fluorescence system (TRXF) while the types and quantities of phytochemicals present were assessed using standard procedures. Aqueous extracts orally and intraperitoneally administered at 50 mg/kg, 100 mg/kg 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 leaf extracts significantly reduced the body weight gain. The same intraperitoneal dose increased the liver and spleen, and decreased the testis weight; and reduced the hemoglobin levels, packed cell volume and increased the platelet count; increased the activity of aspartate aminotransferase, and lactate dehydrogenase, and decreased the activity of alkaline phosphatase, γ-glutamyltransferase, and creatine kinase and histologically slightly injured the liver and spleen and orally increased the activity of alanine aminotransferase, lactate dehydrogenase, and creatine kinase, and decreased the activity of aspartate aminotransferase and γ-glutamyltransferase. The extracts contained phenols, tannins, flavonoids, alkaloids, terpenoids, sterols, and reducing sugars. Potassium, calcium, and iron 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 extract. This study recommends continued use of this plant as an herbal medicine.

Keywords: *Cissampelos pareira;* Leaf extracts; Total Reflection X-ray fluorescence system; Hematological; Biochemical parameters

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. Type 1 diabetes 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). Type 2 diabetes 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 glycated hemoglobin (HbA1C) level (6.1-7.0), fasting (7 mM or greater on two separate occasions) or random blood glucose level (11 mM 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 mM -10.0 mM) [3].

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In conventional medical practice, the present therapies of diabetes mellitus are reported to have side effects. The glucose-lowering drugs include insulin secretagogues (sulfonylureas, meglitinides), insulin sensitizers (biguanides, metformin, thiazolidinediones), α-glucosidase inhibitors (miglitol, acarbose). The peptide analogs, such as exenatide, liraglutide and DPP-4 inhibitors, increase GLP-1 serum concentration and slow down the gastric emptying. 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 acidosis, idiosyncratic liver cell injury, permanent neurological deficit, digestive discomfort, headache, dizziness and even death. Therefore, 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 prevent or cure diseases including diabetes since the dawn of civilization [4].

These plant based herbal medicines 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 *Cissampelos pareira* Linn. *C. pareira* Linn of the family Menispermaceae, is a climbing shrub distributed in the tropical and subtropical warm regions of Asia, Africa (Eastern DR Congo, Tanzania, Kenya, South to North Angola, Zambia, Comoros and Madagascar), and America. In traditional medicine pratice, the roots and leaves of *C. pareira* are used as immunomodulators, diuretics, purgatives, antidyspepsia, antidropsy, anticough, anticancer (antileukemia and gastric cancer), antiashmatic, antifertility, antimalaria, antiseptics, antihelminthic, antibacteria, antifungal, antivenom, antihistaminic, antispasmodic, anticonvulsant, antipyretic, and anti-inflammatory agents. The roots of *C. pareira* are also used to manage heart diseases such as hypertension, irregular heart beat; urinary tract infections; urigental diseases such as menstrual problems, venereal diseases, uterine haemorrage and threatened miscarriages; gastrointestinal complaints such as diarrhoea, dysentery, ulcers, colic and digestive complaints; skin infections such sores, boils, scabies and children eczema; wounds; conjunctivitis and diabetes in many countries including Kenya [5-11].

The compounds isolated from *C. pareira* includes alkaloids such as bebeerine, bebecrine, buxine, cissampareine, cissamine, cycleanine, d-4"-O-methylbebeerine, dehydrodicentrine, dicentrine, grandirubine, haytinin, haytidin, isochondrodendrine, isoimerubrine, isularine, pareirubrine A and B, pareirubrine, pareitropone, and tetrandine; flavone cissampeloflavone; quercetin such as 2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one; fatty acids such as linoleic acid, arachidic acid and stearic acid; sterols such as β-sitosterol and other compounds including bulbocaprine, corytuberine, curine, cyclanoline, dimethyltetrandrinium, laudanosine, magnoflorine, menismine, norimeluteine, norruffscine, nuciferine, and quercitol [6,11-16].

Cissampareine has inhibitory activity against human carcinoma cells of the nasopharynx in cell culture. It is used as a skeletal muscle relaxant. Methiodide and methochloride derivatives of haytinin are potent neuromuscular blocking agents and lower blood pressure. Tetrandrine in low doses is cardioprotective and lowers blood pressure. Berberine has hypotensive, antifungal and antimicrobial actions. It is used to manage irregular heart beat, cancer, Candida, diarrhoea, and irritable bowel syndrome [4,11]. While *C. mucronata* in the same family as *C. pareira* has been reported to demonstrate hypoglycemic activity in streptozocin induced diabetic wistar rats on single intraperitoneal administration of ethanol extracts at 200 mg/kg, 400 mg/kg and 800 mg/kg body weight doses, and its safety confirmed [17], no reported study has been performed on *C. pareira* using water extracts 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 *C. pareira* 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 extracts of *C. pareira* at therapeutic and high doses 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 *C. pareira* were collected in September 2007 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 extracts

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

One hundred grams of powdered leaf 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 g-150 g with a mean weight of 120 g. These were bred in the Animal house at the Department of Biochemistry and Biotechnology, 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-dioxyuracil) obtained from Sigma (Steinhein, 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 hours-12 hours but allowed free acess to water until the end of the experiment [18].

Experimental Design

For either intraperitoneal 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 either intraperitoneally or orally administered with 0.1 ml physiological saline; Group II consisted of alloxan induced diabetic rats either intraperitoneally or orally administered with 0.1 ml physiological saline; Group IIIa consisted of alloxan induced diabetic rats intraperitoneally administered with 0.12 units of insulin (1 IU/kg body weight) in 0.1 ml physiological saline; Group IIIb consisted of alloxan induced diabetic rats orally administered with 0.36 mg of glibenclamide (3 mg/kg body weight) in 0.1 ml physiological saline; Group IV consisted of alloxan induced diabetic rats either intraperitoneally or orally administered with 6 mg extract (50 mg/kg body weight) in 0.1 ml physiological saline; Group V consisted of alloxan induced diabetic rats either intraperitoneally or orally administered with 12 mg extract (100 mg/kg body weight) in 0.1 ml physiological saline; and Group VI consisted of alloxan induced diabetic rats either intraperitoneally or orally administered with 18 mg extract (150 mg/kg body weight) in 0.1 ml physiological saline.

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 hour, 2 hours, 3 hours, 4 hours, 12 hours 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) [19]. The half-life was calculated by substituting for the rate constant (k) in the formulae: $t_{0.5}$=0.693/k where by $t_{0.5}$ is the time when the dosage has reduced the plasma sugar level by half [20]. The exponential decay equation was used to get the dosage that would be administered after a certain period [21].

In Vivo single dose toxicity test

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

Determination of body and organ weight

The body weight of each rat was assessed after every seven days during the dosing period up to and including the 28th day and the day of sacrifice**.** On the day of sacrifice, all the animals were euthanized. The heart, liver, lungs, spleen, kidneys, brain, eyes and testis were carefully dissected out and weighed. These organ were then stored in 10% neutral buffered formalin.

Determination of hematological parameters

Blood parameters and indices were determined using standard protocols [22]. Red blood cells, white blood cells, hemoglobin, mean cell hemoglobin, mean cell hemoglobin concentration and mean cell volume were determined using the Coulter Counter System (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 [22]. 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°C until required for biochemical parameter analysis.

Determination of biochemical parameters

The biochemical parameters determined on the sera specimen using the Olympus 640 Chemistry AutoAnalyser were alanine aminotransferase (AST), aspartate aminotransferase (ALT), alkaline phosphatase (ALP), γ-glutamyl transferase (GGT), lactate dehydrogenase (LDH), blood urea nitrogen (BUN), creatinine (CREAT), amylase (AMY) and creatinine kinase (CK). 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°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 liver, kidney, heart, spleen, brain, lungs, eyes and testis were observed for any histopathological changes.

Determination of *C. pareira* extracts phytochemicals

A phytochemical screening of phenols, alkaloids, flavonoids, saponins, tannins, terpenoids, sterols, cardiac glycosides, phylobatannins, resins, and sterols, and free and bound anthraquinones present in *C. pareira* extracts was performed using standard methods [23,24]. For quantitative determination of phytochemicals, 2 g of the

C. pareira 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 [25], tannins were determined using the method described by [26], alkaloids were determined using the method described by [27] and flavonoids were determined using the method described by [28].

Mineral composition of leaf extracts of *C. pareira* using TXRF system

TXRF system was used to determine the content of potassium, calcium, iron, manganese, copper, zinc and lead in the leaf extracts of *C. pareira*. 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 [29]. 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^2 × 10 mm sensitive volume, 25 μm Be window) with an energy resolution of 200 eV at 5.9 keV Mn K_α - 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 [30] that is based on the fundamental parameters method (FPM) [31,32]. 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 ≈ μg/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 ± standard deviation (SD) of the number of animals used per every study point. Statistical analysis was done using ANOVA and Boniferroni-Holm test 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 mg/kg, 100 mg/kg and 150 mg/kg body weight. $p \leq 0.05$ was considered statistically significant.

Results

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

C. pareira aqueous leaf extract yielded a 4% sticky black paste. Intraperitoneal administration of aqueous leaf extracts of *C. pareira* decreased the blood glucose levels at all the three doses (50 mg/kg, 100 mg/kg 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. However, the sugar levels were not reduced in a dose dependent manner. In the first hour, the extracts lowered blood glucose levels by 42%, 51% and 43% for 50 mg/kg,100 mg/kg and 150 mg/kg body weight doses, respectively, compared to insulin treated diabetic rats whose blood sugar levels was lowered by 40% within the first hour. By the third hour, all the three doses (50 mg/kg, 100 mg/kg and 150 mg/kg body weight) had lowered blood sugar levels by 61%, 63% and 67%, respectively, compared to insulin treated diabetic rats whose sugar levels was lowered by 74% within the same hour.

Oral administration of the aqueous leaf extracts of *C. pareira* also lowered blood glucose levels at all the three doses (50 mg/kg, 100 mg/kg and 150 mg/kg body weight) from the first hour to the fourth hour and again in a dose-independent manner. By the second hour the extract had lowered the blood glucose levels by 55%, 57% and 55%, respectively for the three doses, compared to 63% for the convectional oral drug, glibenclamide. The reduction in blood glucose levels when compared to the negative control was statistically significant ($p \leq 0.05$).

Table 1, 2 shows the pharmacokinetics of the hypoglycemic activity for the first four hours of the aqueous leaf extracts of *C.*

Treatment	Route	Glucose Levels at Varying Times (mg/dL)						
		0hr	1hr	2hr	3hr	4hr	12hr	24hr
Group I	IP	60.50 ± 5.57	59.25 ± 4.99	60.75 ± 5.56	61.25 ± 4.35	58.50 ± 7.19	51.50 ± 1.29	53.00 ± 1.83
	Oral	66.50 ± 9.98	67.25 ± 8.62	69.50 ± 7.05	69.50 ± 5.45^{a}	68.75 ± 6.85^{a}	62.00 ± 9.83^{a}	61.25 ± 9.61
Group II	IP	222.25 ± 11.33^{A}	223.75 ± 11.15^{B}	227.75 ± 10.63^{B}	233.25 ± 11.09^{B}	239.00 ± 11.10^{C}	250.25 ± 15.48^{B}	261.25 ± 15.37^{B}
	Oral	204.25 ± 8.66^{a}	206.25 ± 8.46^{b}	208.00 ± 8.13^{b}	209.75 ± 8.54^{b}	212.00 ± 7.87	218.75 ± 10.21^{c}	227.25 ± 6.65^{c}
Group III	IP	220.25 ± 10.78^{A}	132.00 ± 17.57^{A}	64.25 ± 3.30	57.50 ± 2.65	54.25 ± 1.71	51.25 ± 1.89	51.25 ± 1.71
Group IV	Oral	193.75 ± 22.69^{a}	96.50 ± 10.85^{a}	70.25 ± 7.63	58.75 ± 5.91	51.75 ± 2.22	49.50 ± 1.29	53.50 ± 1.29
Extract								
Group V	IP	215.50 ± 12.66^{A}	123.50 ± 16.70^{A}	94.50 ± 7.42^{A}	83.00 ± 8.60^{A}	74.25 ± 5.38^{A}	97.75 ± 2.99^{A}	114.50 ± 6.35^{A}
	Oral	198.75 ± 18.34^{a}	105.50 ± 4.20^{a}	89.00 ± 3.16^{a}	81.25 ± 1.71^{a}	70.75 ± 1.708^{a}	95.00 ± 4.32^{b}	116.50 ± 4.20^{b}
Group VI	IP	222.00 ± 15.64^{A}	107.75 ± 7.04^{A}	88.00 ± 5.72^{A}	79.75 ± 3.30^{A}	79.25 ± 2.06^{B}	94.25 ± 2.50^{A}	113.75 ± 5.85^{A}
	Oral	190.75 ± 14.59^{a}	102.00 ± 2.58^{a}	79.75 ± 1.71^{a}	78.25 ± 1.50^{a}	77.00 ± 1.83^{a}	90.25 ± 2.22^{b}	102.00 ± 3.74^{a}
Group VII	IP	226.50 ± 11.96^{A}	108.75 ± 5.38^{A}	87.00 ± 3.92^{A}	74.00 ± 4.08^{A}	65.00 ± 1.41	83.50 ± 6.86^{A}	110.00 ± 4.97^{A}
	Oral	204.50 ± 9.98^{a}	104.75 ± 4.86^{a}	91.00 ± 6.16^{a}	80.75 ± 7.41^{a}	72.50 ± 1.83^{a}	89.50 ± 3.70^{b}	111.00 ± 3.56^{a}

Results are expressed as Means ± SEM for four animals per group. Means for intraperitoneally (IP) administered drugs followed by similar upper case letters are not significantly different at p<0.05 by ANOVA and Boniferroni-Holm test; Means for orally (oral) administered drugs followed by similar lower case letters are not significantly different at p<0.05 by ANOVA and Boniferroni-Holm test. Group I represents the normal control rats orally or intraperitoneally administered with 0.1 ml physiological saline; Group II represents diabetic rats orally or intraperitoneally administered with 0.1 ml physiological saline; Group III represents diabetic rats intraperitoneally administered with insulin at 1 IU/kg body weight; Group IV represents diabetic rats orally administered with glibenclamide at 3 mg/kg body weight; Group V represents diabetic rats orally and intraperitoneally administered with aqueous extracts of *C. pareira* at 50 mg/kg body weight; Group VI represents diabetic rats orally and intraperitoneally administered with aqueous extracts of *C. pareira* at 100 mg/kg body weight; Group VII represents diabetic rats orally and intraperitoneally administered with aqueous extracts of *C. pareira* at 150 mg/kg body weight. mg/kg bw stands for mg/kg body weight.

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

Drug (dose)	Route	Rate constant (hour^{-1})	Half-life (hours)
Insulin (1IU/kg bw)	IP	0.3634	1.91
Glibenclamide (3mg/kg bw)	Oral	0.3132	2.21
Extract (mg/kg bw)			
50	IP	0.2432	2.85
	Oral	0.2328	2.98
100	IP	0.2215	3.12
	Oral	0.2081	3.33
150	IP	0.2606	2.66
	Oral	0.2335	2.92

Results are expressed as Means of four rats for each time point; bw represents body weight

Table 2: Pharmacokinetics of the hypoglycemic activity for the first four hours of the three doses of the aqueous leaf extracts of *C. pareira*

pareira. Results indicate that the pseudo-first order rate constants for the three doses of the aqueous leaf extracts of *C. pareira* together with their accompanying half-lifes were similar but lower than those of the insulin and glibenclamide. The rate constants for the aqueous leaf extracts for the three doses intraperitoneally and orally administered ranged from 0.2081 to 0.2606 per hour and the half-lifes ranged from 2.66 to 3.33 hours, respectively. The rate constant for insulin was 0.3634 per hour and that of glibenclamide was 0.3132 per hour while their corresponding half-lifes were 1.91 hours and 2.21 hours, respectively.

Effects of intraperitoneal and oral administration of aqueous extracts of *C. pareira* at 1 g/kg body daily in rats for 28 days in rats on body weight and gain in body weight

As depicted in Table 3, oral and intraperitoneal administration of aqueous extracts of *C. pareira* at 1g/kg body weight in rats for 28 days daily significantly decreased growth rate measured in terms of body weight gain and weekly change in body weight relative to the control rats. Initially for the orally treated rats, the control and *C. pareira* treated rats had similar body weights which continued up to the 14th day. Thereafter, the control rats grew at a significantly greater rate than the *C. pareira* treated rats. For the intraperitoneally treated rats, the control rats had a significantly lower body weight than the *C. pareira* treated rats. This continued up to the 14th day when both the control and *C. pareira* treated rats had similar body weight. Thereafter, the control rats grew at a significantly greater rate than the *C. pareira* treated rats.

Effects of oral and intraperitoneal administration of aqueous extracts C. pareira at 1 g/kg body weight daily in rats for 28 days on the percent relative organ weight

As depicted in Table 4, oral administration of aqueous plant extracts of *C. pareira* at 1 g/kg body weight daily in rats for 28 days significantly increased the percent relative organ weight of the kidney relative to the control rats. In addition, intraperitoneal administration of the aqueous extracts of *C. pareira* at the same dose in rats daily for 28 days significantly increased the percent relative organ weight of the liver, kidney, lungs, heart, spleen, and eyes relative to that of the control rats.

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

Intraperitoneal administration of aqueous leaf extracts of *C. pareira* at 1 g/kg body weight daily to rats for 28 days significantly decreased the levels of haemoglobin, packed cell volume and significantly increased the levels of platelets; however, it had no significant effect on red blood cell count, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and mean cell volume (Table 5). This intraperitoneal dose had no significant effect on the white blood cells, neutrophils, lymphocytes, eosinophils and monocytes (Table 5; Table 6). Oral administration of aqueous leaf extracts of *C. pareira* at 1g/kg body weight daily to rats for 28 days had no effect on all the measured hematological parameters and the differential white blood cell count (Table 5; Table 6).

Effects of intraperitoneal and oral administration of aqueous leaf extracts of *C. pareira* at 1 g/kg body weight daily in rats for 28 days on some end point biochemical parameters

Intraperitoneal administration of aqueous leaf extracts of *C. pareira* at 1g/kg body weight daily to rats for 28 days significantly increased the activities of aspartate aminotransferase (AST), and lactate dehydrogenase (LDH), and decreased the activities of alkaline phosphatase (ALP), γ-glutamyltransferase (GGT), and creatine kinase (CK); however, it had no significant effect on the activities of alanine aminotransferase (ALT), and amylase (AMY) (Table 7). Oral administration of aqueous leaf extracts of *C. pareira* at 1g/kg body weight daily to rats for 28 days significantly increased the activities of alanine aminotransferase (ALT), lactate dehydrogenase (LDH) and decreased the activities of aspartate aminotransferase (AST) and γ-glutamyltransferase (GGT); however, it had no significant effect on the activities of alkaline phosphatase (ALP) and amylase (AMY) (Table 7).

Histopathology

Oral administration of aqueous leaf extracts of *C. pareira* at 1 g/kg body weight daily in rats for 28 days showed no gross or histological pathology in any of the organs. However, intraperitoneal administration of aqueous leaf extracts of *C. pareira* at 1 g/kg body weight daily in rats for 28 days in rats resulted in mild histological changes in the spleen and liver (Figures 1 and 2). The spleen sections of rats intraperitoneally administered with aqueous leaf extracts of *C. pareira,* showed reduction in cell density of lymphoid follicles (Figure 3) when compared with controls (Figure 4). The liver of rats intraperitoneally treated with aqueous extracts of *C. pareira* showed changes in the peritoneal lining. The serosa lining of the liver was inflamed with proliferation of fibrous tissue and presence of mixed inflammatory cells, indicating presence of peritonitis (Figure 5); the hepatocytes adjacent to the affected peritoneum were degenerated and the normal hepatic columns were absent when compared with those in normal liver (Figure 6 and Figure 7).

Qualitative and quantitative phytochemical composition of aqueous leaf extracts of *C. pareira*

Results show that aqueous extracts of *C. pareira* contained detectable levels of phenols, alkaloids, flavonoids, tannins, terpenoids, sterols and reducing sugars. The aqueous leaf extracts of *C. pareira* lacked detectable levels of saponins, phylobatannins, cardiac glycosides, resins and free and bound anthraquinones. Quantitatively, the levels of phenols and tannins were 660.25 mg/g ± 35.53 mg/g and 342.90 mg/g ± 33.94 mg/g Gallic equivalent, respectively, while that of flavonoids and alkaloids were 138.67 mg/g ± 28.70 mg/g and 50.00 mg/g ± 7.21 mg/g, respectively.

Treatment	Weekly Weight of Rats in g					Δ Weight/Week
	0	1	2	3	4	
Control (oral)	142.7 ± 2.5	148.0 ± 0.0	153.7 ± 1.5	166.3 ± 5.5	174.0 ± 5.0	7.8 ± 0.6
C. pareira	143.0 ± 4.4	145.3 ± 3.2	149.7 ± 1.5	154.0 ± 2.0*	157.3 ± 2.5*	3.6 ± 0.5*
Control (IP)	136.0 ± 6.6	147.0 ± 5.6	168.3 ± 8.5	191.0 ± 2.0	203.0 ± 6.1	16.8 ± 0.1
C. pareira	161.7 ± 9.1*	167.7 ± 7.5*	169.3 ± 7.5	171.7 ± 7.8*	172.7 ± 7.6*	2.8 ± 0.4*

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 administration of aqueous extracts *C. pareira* at 1 g/kg body weight daily in rats for 28 days on body weight

Treatment	Percent Relative Organ Weight (g/100g)							
	Liver	Kidney	Lungs	Heart	Testis	Brain	Spleen	Eyes
Control (Oral)	5.30 ± 0.31	0.92 ± 0.02	1.37 ± 0.23	0.49 ± 0.07	1.73 ± 0.11	1.17 ± 0.07	0.62 ± 0.06	0.22 ± 0.04
C. pareira	5.09 ± 0.19	0.98 ± 0.02*	1.07 ± 0.10	0.47 ± 0.01	1.79 ± 0.15	1.25 ± 0.03	0.75 ± 0.08	0.26 ± 0.02
Control (IP)	4.73 ± 0.22	0.84 ± 0.03	1.07 ± 0.08	0.38 ± 0.03	1.34 ± 0.11	1.17 ± 0.09	0.40 ± 0.05	0.14 ± 0.01
C. pareira	9.48 ± 0.41*	1.07 ± 0.11*	1.58 ± 0.11*	0.50 ± 0.02*	1.10 ± 0.10	1.28 ± 0.09	1.06 ± 0.11*	0.16 ± 0.01*

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 4: Effects of oral administration of aqueous extracts of *C. pareira* at 1 g/kg body weight daily in rats for 28 days on the percent relative organ weight

Treatment	Hematological Parameters						
	Hb (g/dL)	RBC (× 10^{12}/L)	PCV (%)	MCH (pg)	MCHC (g/dl)	MCV (fL)	PLT (x10^9/l)
Control (Oral)	12.40 ± 0.61	6.18 ± 0.20	37.53 ± 1.76	20.07 ± 0.45	33.03 ± 1.12	60.73 ± 2.44	469.33 ± 71.52
C. pareira	12.60 ± 0.30	6.14 ± 0.56	37.27 ± 1.50	20.60 ± 1.37	33.83 ± 0.61	60.90 ± 3.10	309.33 ± 69.76
Control (IP)	14.60 ± 0.40	7.61 ± 0.66	44.27 ± 1.75	19.23 ± 1.22	33.00 ± 0.40	58.37 ± 3.30	370.33 ± 68.77
C. pareira	10.90 ± 0.56*	5.92 ± 0.20	33.50 ± 1.35*	18.40 ± 0.56	32.50 ± 0.36	56.60 ± 1.37	610.00 ± 50.19*

Results are expressed as Mean ± Standard Deviation (SD) for four animals in each treatment; *p<0.05 is considered significant when the mean of the control animals is significantly different from that of the extract treated animals by t-test.

Table 5: Effects of intraperitoneal and oral administration of aqueous leaf extract of *C. pareira* at 1 g/kg body weight daily in rats for 28 days on hematological parameters.

Treatment	Absolute differential white blood cell count				
	WBC (× 10^9/l)	Neutrophils (× 10^9/l)	Lymphocytes (× 10^9/l)	Eosinophils (× 10^9/l)	Monocytes (× 10^9/L)
Control (Oral)	6.80 ± 1.26	4.26 ± 0.56	1.87 ± 0.57	0.38 ± 0.08	0.29 ± 0.07
C. pareira	7.39 ± 1.56	4.42 ± 1.06	1.99 ± 0.41	0.56 ± 0.09	0.41 ± 0.04
Control (IP)	8.04 ± 1.08	4.70 ± 0.52	2.37 ± 0.35	0.57 ± 0.20	0.40 ± 0.07
C. pareira	10.62 ± 1.95	6.51 ± 1.27	2.74 ± 0.53	0.78 ± 0.13	0.59 ± 0.08

Results are expressed as Mean ± Standard Deviation (SD) for four animals in each treatment; *p<0.05 is considered significant when the mean of the control animals is significantly different from that of the extract treated animals by t-test.

Table 6: Effects of intraperitoneal and oral administration of aqueous leaf extracts of *C. pareira* at 1 g/kg body weight daily in rats for 28 days on white blood cells (WBC) and the absolute differential white blood cell count

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)	26.70 ± 6.40	134.00 ± 21.20	16.30 ± 0.60	41.70 ± 13.90	140.0 ± 13.9	108.0 ± 13.2	92.7 ± 6.0
C. pareira	63.70 ± 15.20*	63.30 ± 4.20*	15.00 ± 1.00	14.30 ± 4.0*	262.3 ± 21.8*	124.3 ± 9.5*	110.0 ± 12.1
Control (IP)	28.7 ± 4.2	176.3 ± 24.4	26.7 ± 3.1	36.0 ± 7.2	177.0 ± 29.5	169.7 ± 24.0	79.3 ± 5.8
C. pareira	50.7 ± 10.0	394.0 ± 18.0*	15.0 ± 1.0*	14.3 ± 4.0*	371.0 ± 73.6*	82.3 ± 8.6*	144.3 ± 31.5

Results are expressed as Mean ± Standard Deviation (SD) for four animals in each treatment; *p<0.05 is considered significant when the mean of the control animals is significantly different from that of the extract treated animals by t-test.

Table 7: Effects of intraperitoneal and oral administration of aqueous leaf extracts of *C. pareira* at 1 g/kg body weight daily in rats for 28 days on biochemical parameters.

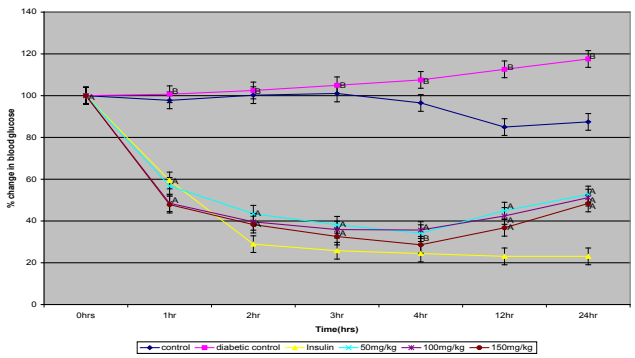

Figure 1: Mean percentage change in blood glucose levels induced by intraperitoneal administration of 50 mg/kg, 100 mg/kg and 150 mg/kg body weight dose of leaf extracts of *C. pareira* in alloxan induced diabetic rats. Values are expressed as % Means ± SEM for four rats for each time point. Means for intraperitoneally (IP) administered drugs followed by similar upper case letters are not significantly different at p<0.05 by ANOVA and Boniferroni-Holm test.

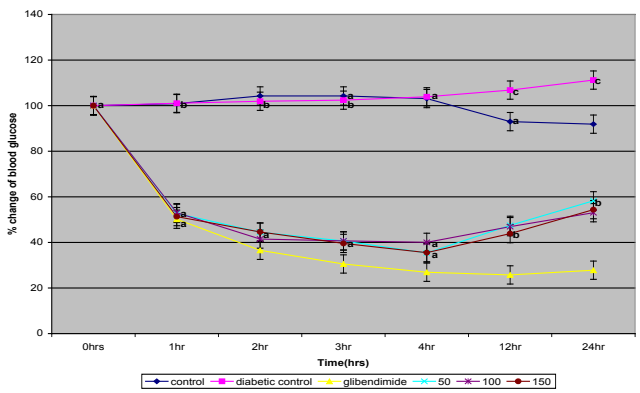

Figure 2: Mean percentage change in blood glucose levels induced by oral administration of 50 mg/kg, 100 mg/kg and 150 mg/kg body weight dose of leaf extracts of *C. pareira* in alloxan induced diabetic rats. Values are expressed as % Means ± SEM for four rats for each time point. Means for intraperitoneally (IP) administered drugs followed by similar lower case letters are not significantly different at p<0.05 by ANOVA and Boniferroni-Holm test.

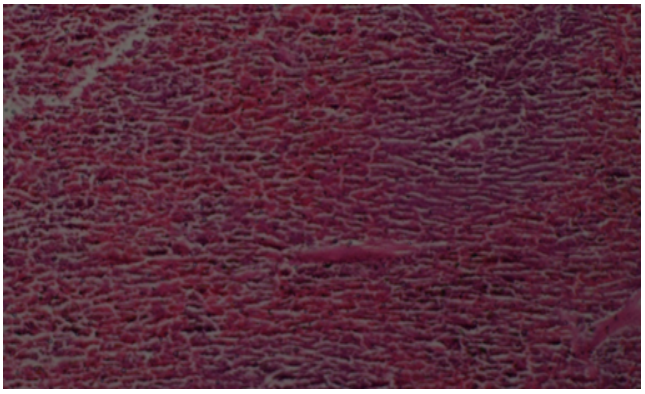

Figure 3: Histological section of a spleen of a rat treated intraperitoneally with an aqueous leaf extract of *C. pareira* (1 g/kg body weight/day) for 28 days. There is reduction in cell density in the lymphoid follicles (arrow); Magnification × 100

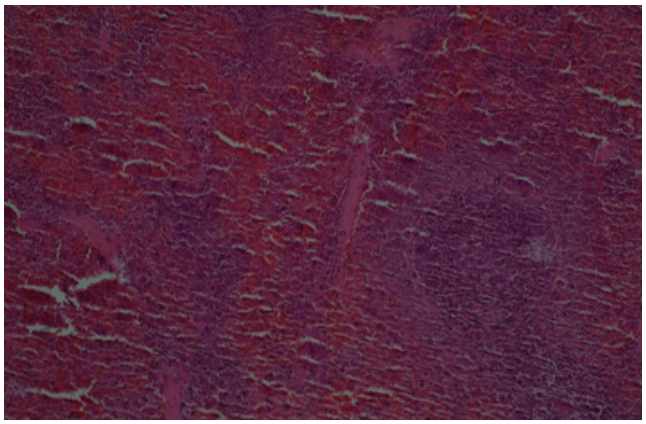

Figure 4: Histological section of a spleen from a normal rat treated intraperitoneally with physiological saline daily for 28 days. The lymphoid follicles are intact and well-populated (arrow); Magnification × 100.

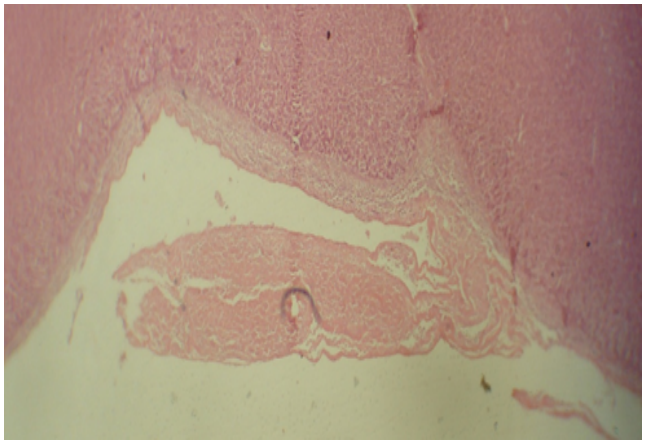

Figure 5: Histological section of a liver of a rat treated intraperitoneally with an aqueous leaf extracts of *C. pareira* (1 g/kg body weight/day) daily for 28 days. The serrosa is lined with a proliferative inflammation that indicates peritonitis (arrow); Magnification × 100

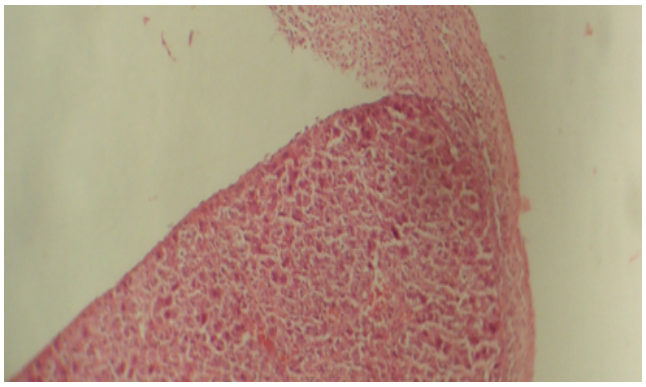

Figure 6: Histological section of a liver of a rat intraperitoneally administered with aqueous leaf extracts of *C. pareira* (1 g/kg body weight/day) daily for 28 days. Lining of the liver is inflamed (arrow). The hepatocyte columns are lost and some cells appear shrunken with a larger nucleus that appears like a dark stain; Magnification: × 100.

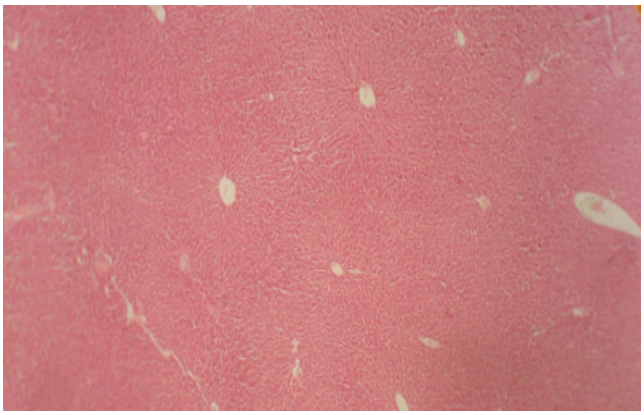

Figure 7: Histological section of a liver from a normal rat intraperitoneally administered with physiological saline daily for 28 days. The liver cells are compact including those at the lining; Magnification: × 100.

Minerals composition of the aqueous leaf extracts of *C. pareira* (µg/g) and the quantity of each mineral in 1 g plant extracts per kg body weight orally and intraperitoneally administered to each rat per day (µg/day)

Table 8 shows the minerals composition of the aqueous leaf extracts of *C. pareira* (µg/g) and the quantity of each mineral administered in 1 g plant extracts per kg body weight to each rat per day (µg/day). Results show that the aqueous leaf extracts of *C pareira* contained detectable levels of potassium (K), calcium (Ca), and iron (Fe) and undetectable quantities of manganese (Mn), copper (Cu), zinc (Zn) and lead (Pb). In addition, these results indicate that the extracts provided potassium, calcium and iron at levels below the recommended daily allowance.

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 pancreatic β-cells via formation of reactive oxygen species like nitric oxide [18]. The aqueous leaf extract of *C. pareira* showed blood glucose lowering effect in a dose independent manner when administered intraperitoneally and orally indicating that they contained hypoglycemic constituents. The lowering effect of blood sugar levels by *C. pareira* in a dose independent manner may reflect uptake of the active constituents through saturable active transport, or it may also reflect maximum hypoglycemic activity at the lowest dose used (50 mg/kg body weight). The greater glucose disappearance rate and a short half-life of this extract observed after intraperitoneal administration relative to the oral route could be associated with the immediate higher bioavailability of active constituents to the systemic circulation while in the oral route the active constituents concentration could either have been reduced before reaching the systemic circulation through the first-pass liver metabolism or had limited gastrointestinal absorption via the gut mucosal epithelial cells. The swallowed active constituents are absorbed by the gut mucosal epithelial cells and enter the hepatic portal system which transports them through the portal vein into the liver where substantial amounts are metabolized before release of reduced levels of un-metabolized active constituents into systemic circulation [33,34].

The blood glucose lowering effect of this plant extracts may be attributed to the presence of phenols, flavonoids, alkaloids, tannins, terpenoids and sterols that have been associated with hypoglycemic activity [35]. The presence of alkaloids, flavonoids, and saponins has been reported in chloroform extracts of *C pareira* by [36]. As reported by [37], flavonoids like myricetin, a polyhydroxylated flavonol has insulinomimetic properties and stimulate lipogenesis and glucose transport in the adipocytes hence lowering blood sugar [35]. Similar studies done on *Pterocarpus marsupium* found epicatechin and catechin flavonoids having anti-diabetic properties [38].

The alkaloids present in the aqueous leaf extract of this plant have also been reported to have antihyperglycemic activity. Alkaloids such as bebecrine, hyatin, tetrandine, berberine, bebeerine, cissampareine, cissamine, pareirubrine A and B, buxine, pareitropone, and trandrine have been reported in *C. pareira* [6,12-16]. Alkaloids berberine and tetrandine have been reported to demonstrate antioxidant activity and this activity could be responsible for the various biological activities associated with this plant including antidiabetic 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 [35]. The aqueous leaf extracts of the same plant contained tannins that are known to have hypoglycemic activity. The tannin epigllo-catechin-3-gallate exhibits antidiabetic activity as demonstrated by [39].

The aqueous leaf extracts of *C. pareira* 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 [40]. Terpenoids also strengthen the skin, increase the concentration of antioxidants in wounds, and restore inflamed tissues by increasing blood supply [40]. Terpenoids also improve lung function [40] and therefore make C. *pareira* 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 [41]. The steroids present in this plant make it a good source of steroidal compounds which are potent precursors for the synthesis of sex hormones [42,43].

Mineral	Mineral levels in the leaf extracts (µg/g)	Daily mineral administered (µg/day)	RDA for rats/day (µg/day)
K	126 ± 11 × 10^2	1512 ± 132	2.0 × 10^6
Ca	696 ± 50	83.5 ± 6.0	1.0 × 10^6
Mn	<100	<12	2.3 × 10^3
Fe	9.6 ± 0.8	1.2 ± 0.1	8.0 × 10^3
Cu	<20	<2.4	1.5 × 10^3
Zn	<10	<1.2	1.1 × 10^4
Pb	<5	<0.6	-

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

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

The presence of phenolic compounds in the leaf extracts of *C. pareira* indicates its antimicrobial properties against pathogenic bacteria [44,45].

The hypoglycemic effect of this plant extract could also be attributed to the presence of iron [46]. Iron influences glucose metabolism and reciprocally, iron influences insulin action. Iron interferes with insulin inhibition of glucose production by the liver [47].

Because the toxicity of a drug to the host cells could render it unsuitable for therapeutic purposes, the toxicity of a high dose of this plant extract was assessed in rats. Oral and intraperitoneal administration of aqueous plant extracts of *C. pareira* at 1 g/kg body weight daily in rats for 28 days resulted in a significant reduction of body weight (9.6%-35.8%) and weekly body weight gain and significant increase in some organs as expressed as percent organ weight to that of the control rats indicating the presence of constituents in the extracts that retard growth. A loss of body weight of more than 10% is associated with protein-energy malnutrition which is associated with impaired physiological function such as impaired cell mediated and humoral immunity. Weight loss of more than 20% is associated with severe protein-energy malnutrition and is associated with pronounced organ dysfunction. Such constituents may include components of terpenoids, alkaloids, and flavonoids present in these extracts in addition to other undetermined constituents.

Alkaloids such as ρ-octopamine and synephrines may reduce body weight by exerting adrenergic agonist activity [48]. Synephrines increase energy expenditure (EE) (resting energy expenditure [by 70%], thermic effect of feeding [by 10%], and energy expenditure of physical activity [by 20%]) and decrease food intake in addition to decreasing gastric motility (slows gastric empting and intestinal transit) [49,50] and indirectly producing increased feeling of satiety and a decreased appetite. Nicotine an alkaloid induces weight loss by exerting its effects through the central nervous system and metabolic actions by reducing appetite and altering feeding patterns. It increases metabolic rate and increases energy expenditure (EE) and hence decreases metabolic efficiency [49,51]. In the CNS, nicotine modulates the central nervous system pathways that regulate several aspects of food intake [49].

The flavonoid, chlorogenic acid reduces body weight by inducing reduction in body fat by reducing the absorption of glucose (energy source) leading to an increase in the consumption of fat reserves. A major consequence of blocking digestion of carbohydrates in the proximal gut is colonic fermentation which leads to increased microbial production of gas in the bowl; gas production limits glucose utilization [49,52]. Catechins (flavonoids) such as epigallocatechin gallate, epigallocatechin, epicatechin gallate, and epicatechin are associated with increase in sympathetic nervous system activity, thermogenesis, and fat oxidation and hence accounting for the reduced body weight [53,54]. Catechins also induce reduction of body fat by inhibiting small intestine micelle formation and inhibiting α-glucosidase activity leading to decreased carbohydrate absorption [49,55]. Caffeine, a flavonoid induces weight loss by decreasing energy intake (EI) [56] by acting through the central and peripheral nervous system mechanisms and by promoting thermogenesis and lipolysis [49,57]. The central nervous system effects of caffeine are due to its effects on the widely distributed adenosine α1, α2A, and α2B receptors [58,59].

Terpenoids such as forskolin, a diterpene reduces body weight by acting on adenylate cyclase that converts ATP to cyclic adenosine monophosphate (cAMP). Cyclic adenosine monophosphate (cAMP) promotes lipolysis, increases the body's basal metabolic rate and increases use of body fat [60], and protein degradation and/or decreases protein synthesis.

For the oral route, an additional mechanism may also account for the observed decrease in weight. The tannins which are present in high amounts in these plants extracts may 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. Digestibility reduction negatively influences intake because of the filling effect associated with undigested feedstuff. 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 and necrosis of the liver and kidney.

Protanthocyanidins (PAs) (condensed tannins) retard growth by inhibiting feed intake and digestibility. Protanthocyanidins (PAs) which are not absorbed by the digestive tract, damage the mucosa of the gastrointestinal tract, decreasing the absorption of nutrients such as proteins, carbohydrates and essential amino acids such as methionine and lysine. They also increase excretion of proteins and essential amino acids and alter the excretion of certain cations [61].

Protein-energy malnutrition causes structural and functional deterioration in several organs including the liver, kidney, lungs, heart, brain, spleen and eyes. Specific organ (s) enlargement after intraperitoneal treatment with an aqueous extract of *C. pareira* at 1 g/kg body weight daily in rats for 28 days may partly be explained by leaky cell membranes which allow the movement of potassium and other intracellular ions into the extracellular space. The increased extracellular load in the interstitium causes water movement and edema in the affected organs. Injury to specific organs or tissues may partly account for increased levels of serum enzyme activities of rats treated with aqueous plants extracts at 1 g/kg body weight daily for 28 days. Injury to liver is supported histologically by inflammation of the liver lining, with proliferation of fibrous tissue and presence of mixed inflammatory cells on the serosa of the liver and loss of hepatocyte cytoplasm and columns in rats intraperitoneally administered with 1g of aqueous extracts of *C. pareira* per kg body weight daily for 28 days.

A daily administration of 1g of the aqueous extracts of *C. pareira* per kg body weight intraperitoneally for 28 days caused a significant decrease in hemoglobin levels and packed cell volume values but other hematological parameters remained largely unchanged and thus inducing normocytic normochromic anemia. This abnormal blood conditions could have been caused by toxic constituents in the plant extracts such as alkaloids, and flavonoids present in this extracts which have previously been reported to reduce erythron parameters [62] ; it could also be due to excessive water intake (overhydration). Reduction in hemoglobin levels as observed after intraperitoneal administration of aqueous extracts of *C. pareira* at a dose level of 1 g/kg body weight to rats daily 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) [63]. 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 [63]. Injury of organs resulting from tissue hypoxia may account for the increased size of the liver, kidney, lungs, heart, spleen and eyes.

Although injury of organs resulting from tissue hypoxia was not histologically demonstrated in this study, it is possible that subcellular damage in organs may account for the altered serum levels of alkaline phosphatase (liver, kidney and spleen), alanine (liver) and aspartate aminotransferase (liver, kidneys, heart and pancreas), γ-glutamyltransferase (liver), lactate dehydrogenase (liver, kidney and heart), creatine kinase (heart and skeletal muscle), and amylase (pancreas) in rats orally and intraperitoneally administered daily with 1g of extracts of *C. pareira* per kg body weight for 28 days [64-67].

The cause of increased platelet count (thrombocytosis) in rats intraperitoneally administered with 1 g of aqueous extracts of *C. pareira* per kg body weight daily for 28 days may be associated with a blood disease such as abnormal bleeding induced by toxic phytochemical substances such as tannins in the plant extract.

The decreased levels of white blood cell count and decreased neutrophil, lymphocytes, eosinophils and monocytes observed in rats orally and intraperitoneally administered with 1g of aqueous plant extracts of *C. pareira* per kg body weight daily for 28 days indicate either liver or spleen or bone marrow injury caused by toxic phytochemicals contained in the extracts. Liver and spleen injury is supported by the observed histopathological changes in rats intraperitoneally administered with 1 g of the same extracts per kg body weight daily for 28 days. The spleen histopathological changes involved lymphoid follicles depopulation, and unfilled/empty sinusoids, while the liver histopathological changes involved inflammation of the liver lining with proliferation of fibrous tissue and presence of mixed inflammatory cells on the serosa of the liver, loss of hepatocyte cytoplasm and columns in rats intraperitoneally administered with 1 g of aqueous extracts of *C. parreira* per kg body weight daily for 28 days. The reduced levels of white blood cell count imply a reduced ability of the body to respond to infection [68,69].

The observation that orally administered *C. pareira* extracts at a high dose caused no histopathological abnormalities compared to the intraperitoneally administered extracts confirms the known fact of the lesser toxicity of drugs administered orally due either to poor absorption, protein binding or metabolism in the gastrointestinal tract. The observation of biochemical abnormalities of the liver, kidney, heart and skeletal muscle of rats intraperitoneally administered with a high dose of this plant extract without obvious gross or histopathological abnormality with extracts administered orally displays the poor diagnostic capacity of histopathology in subclinical toxicity.

In conclusion, *C. pareira* used traditionally in the practice of herbal medicine has demonstrated antidiabetic activity when therapeutic doses were administered intraperitoneally and orally. The intraperitoneal route was more effective than the oral route as assessed from the rate of glucose reduction and its half-life. A very high dose of *C. pareira* (1 g/kg body weight) that is far from the therapeutic dose is likely to produce toxicological effects as assessed by body weight gain and weekly change in body weight, percent relative organ weight, hematological and biochemical parameters and histopathological changes on the liver and spleen. The intraperitoneal route resulted in a higher toxicity compared to the oral route. This may explain why the oral route is the preferred route in ethnopharmacological use in herbal medical practice. The antidiabetic activity and the toxicity of a high dose of this plant may be accounted for by the phytochemicals present in this plant.

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