

Hypoglycemic Effect of Aqueous and Ethyl Acetate Leaf Extract of *Maytenus putterkloides* in Alloxan Induced Diabetic Mice

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Abstract

Maytenus putterkloides has been used traditionally to manage several diseases including diabetes, however, its efficacy has not been scientifically evaluated. The aim of this study was to determine the *in vivo* hypoglycemic activity of aqueous and ethyl acetate leaf extract of this plant in male swiss white albino mice. The antidiabetic activity was screened in alloxan induced diabetic mice through oral and intraperitoneal routes. Phytochemical composition was assessed using standard procedures. The aqueous and ethyl acetate leaf extract of this plant showed hypoglycemic activity at dose levels of 50, 100, 200, 300 mg/kg body weight in a dose independent manner via both routes. The extracts contained total phenolics, saponins, tannins, flavonoids, alkaloids and phytonutrients; vitamins B1, B3, and C. The observed hypoglycemic activity could be associated with the phytochemicals present in this plant extract.

Keywords: Diabetes mellitus; *Maytenus putterkloides*; Hypoglycemic activity; Antidiabetic; Alloxan

Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia and glucose intolerance, either as a result of insulin deficiency or impaired effectiveness of insulin's action, or to a combination of both factors [1]. The prevalence of diabetes is increasing rapidly worldwide and it has been predicted that by 2030 the number of adults with diabetes would have almost doubled worldwide, from 177 million in 2000 to 370 million [2].

Diabetes mellitus may present with characteristic symptoms such as thirst, polyuria, blurring of vision, and weight loss [3]. In its most severe forms, ketoacidosis or a non-ketotic hyperosmolar state may develop and lead to stupor, coma and, in absence of effective treatment, death. Among the complications associated with diabetes mellitus include microvascular complications which mainly affect the retina, kidney and peripheral nervous system and may progress to more overt serious complications, and macrovascular complications, mainly atherosclerosis, that may lead to cerebrovascular ischemia and stroke [3,4].

Normal fasting plasma glucose levels ranges between 3.5-6.7 mmol/l (63-120.6 mg/dl). After a meal the blood glucose level rises to approximately 8 mmol/l and rarely exceeds this level. Repeated fasting blood glucose levels ≥ 7.0 mmol/l (126 mg/dl) or 2-hour postprandial glucose values ≥ 11.1 mmol/l (200 mg/l) is considered to be diagnostic criteria for diabetes and correlates with HbA1c threshold of 6.5% [3]. Currently available therapy for diabetes include insulin and various oral antidiabetic agents such as sulphonylureas, metformin, α -glucosidase inhibitors, troglitazone [5]. These drugs are used as monotherapy or in combination to achieve better glycemic control. However, the use of the above oral agents causes a number of serious adverse

effects which includes digestive disorders (nausea, vomiting), blood disorders (hemolytic anemia) and hypoglycemia [6]. As a consequence there continues to be the high demand for new oral anti-diabetic drug with little or no side effect from herbal sources [7,8].

According to World Health Organization (WHO), up to 80% of the world's population in developing countries relies on traditional medicine practices for their primary health care needs [9]. Plants have always been used to treat many diseases throughout the world. They contain a great diversity of bioactive compounds which makes them a possible source for different types of drugs [10]. Plants are well known in traditional herbal medicine for their hypoglycemic activities and available literatures indicate that there are more than 800 plant species showing hypoglycemic activity [11]. For example the widely used hypoglycemic drug metformin is originally derived from the medicinal plant *Galega officinalis* [12]. There has been increasing demand for the use of plant products with anti-diabetic activity due to its low cost, easy availability and less side effects. Therefore plant materials are continuously scrutinized and explored for their effects as hypoglycemic agents [13]. In the folkloric set up, hot water extract of *M. putterkloides* has been reported to manage diabetes mellitus; however, there is no documented scientific investigation that has been conducted to ascertain the validity of this claim. Therefore, this study was carried out to determine the *in vivo* hypoglycemic activity of aqueous and ethyl acetate leaf extract of *M. putterkloides* in alloxanized diabetic male mice.

Materials and methods

Study site

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

Collection of the plant materials

The plant used in this study was collected from its natural habitat in Siakago village, Embu County, Kenya. An acknowledged authority in taxonomy authenticated the botanical identity of the plant and a voucher specimen was deposited at the National Museums of Kenya Herbarium, Nairobi. A traditional medical practitioner provided the information on which plant to collect, what part to collect, and the precise locality where it grows.

The collected plant leaves were dried under shed at room temperature for 1 month, and then ground into powder using an electric mill. The powdered plant material was kept at room temperature away from direct sunlight in closed dry plastic bags.

Preparation of the aqueous extract

One hundred grams of the powdered plant material was extracted in 1 liter distilled water at 60°C for 6 hr. The mixture was left to cool at room temperature and then decanted into dry clean conical flask through folded cotton gauze stuffed into a funnel. The decanted extract was then filtered using filter papers under vacuum pump. The filtrate was then freeze-dried for 72 hr. The freeze-dried powder was then weighed and stored in airtight container at -20°C until used for bioassay.

Preparation of the ethyl acetate extract

Similarly, 100 g of leaf powder was extracted in 1 liter of ethyl acetate at room temperature in a metabolic shaker for six hours. The extracts were filtered with a Whatman filter No 540 and the filtrate was evaporated in rotary evaporator. The extract was weighed and stored in airtight, amber containers at 4°C ready for use.

Experimental animals

The study used male Swiss White Albino mice (3-6 weeks old) that weighed 23-27 g with a mean weight of 25 g. These were bred in the Animal house at the Department of Biochemistry and Biotechnology of Kenyatta University. The mice were housed at a temperature of 25°C with 12 hr/12 hr 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.

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 (Steinheim, Switzerland) [6]. 48 hr after alloxan administration, blood glucose level was measured using a glucometer. Mice with blood glucose levels above 200 mg/l (or ≥ 11.1 mmol/l) were considered diabetic and used in this study. Prior to initiation of this experiment, the animals were fasted for 8-12 hr [14] but allowed free access to water until the end of this experiment.

Experimental design

For either intraperitoneal or oral route of drug administration, the experimental mice were randomly divided into seven groups of five animals each. Group I consisted of normal mice either intraperitoneally or orally administered with 0.1 ml physiological saline; Group II consisted of alloxan induced diabetic mice either intraperitoneally or orally administered with 0.1 ml physiological

saline; Group IIIa consisted of alloxan induced diabetic mice intraperitoneally administered with 0.025 insulin units (0.25 insulin units in 1 ml) (1 IU/kg body weight) in 0.1 ml physiological saline; Group IIIb consisted of alloxan induced diabetic mice orally administered with 0.075 mg glibenclamide (0.75 mg in 1 ml) (3 mg/kg body weight) in 0.1 ml physiological saline; Group IV consisted of alloxan induced diabetic mice either intraperitoneally or orally administered with 1.25 mg extract (12.5 mg in 1ml physiological saline) (50 mg/kg body weight) in 0.1 ml physiological saline; Group V consisted of alloxan induced diabetic mice either intraperitoneally or orally administered with 2.5 mg extract (25 mg extract in 1 ml physiological saline) (100 mg/kg body weight) in 0.1 ml physiological saline; Group VI consisted of alloxan induced diabetic mice either intraperitoneally or orally administered with 5 mg extract (50 mg extract in 1 ml physiological saline) (200 mg/kg body weight) in 0.1 ml physiological saline; Group VII consisted of alloxan induced diabetic mice either intraperitoneally or orally administered with 7.5 mg extract (75 mg extract in 1 ml physiological saline) (300 mg/kg body weight) in 1 ml physiological saline. 0.1 ml of either insulin or glibenclamide or the plant extract solution was administered either intraperitoneally or orally to each experimental mouse.

Blood sampling and blood glucose 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 2, 4, 6, 8 and 24 hr. Bleeding was enhanced by gently "milking" the tail from the body towards the tip. After the operation, the tips of the tail were sterilized by swabbing with 70% ethanol. The blood glucose levels were determined with a glucose analyser model (Hypogaurd, Woodbridge, England).

Phytochemical studies

The extract was analyzed for the following phytochemicals; total phenolics, saponins, tannins, flavonoids, alkaloids and phytonutrients; vitamins B1, B3, and C according to the method described by Roy, 2015 [15].

Statistical analysis

All the data was recorded as mean \pm standard deviation (SD) of the blood glucose levels. One-way ANOVA and post-ANOVA (Tukey's post hoc test) was used to compare the means of untreated normal control mice with diabetic mice treated with saline, diabetic mice treated with the conventional drug, and diabetic mice treated with plant extracts at doses of 50, 100 and 200 mg per kg body weight. $P \leq 0.05$ was considered statistically significant.

Results

Effect of oral and intraperitoneal administration of aqueous leaf extract of *M. putterkloides* on blood glucose levels in alloxan induced diabetic mice

The dry powder of *M. putterkloides* yielded 5% (w/w) aqueous leaf extract. Treatment with the aqueous leaf extract showed hypoglycemic properties in alloxan induced diabetic mice (Tables 1 and 2; Figures 1 and 2). Oral administration of the extract lowered the blood glucose levels at all the four tested dose levels (50, 100, 200 and 300 mg/kg body weight) in a dose independent manner. As shown in Figure 2, the

aqueous leaf extract of *M. putterkloides* decreased the blood glucose levels appreciably by the four therapeutic doses from the 2nd to the 8th hour. In the 24th hour the plant extract had lost its hypoglycemic effect and the animals started regaining their diabetic states.

Treatment Groups	0 hr	2 hr	4 hr	6 hr	8 hr	24 hr
Normal Control	5.12 ± 0.04	5.16 ± 0.02 ^d	5.14 ± 0.04 ^b	5.12 ± 0.02 ^b	5.12 ± 0.04 ^b	5.12 ± 0.04 ^c
Diabetic control	15.26 ± 1.14	16.66 ± 1.31 ^a	17.58 ± 1.33 ^a	19.18 ± 1.42 ^a	19.98 ± 1.43 ^a	23.46 ± 1.00 ^a
Reference group	14.80 ± 1.40	7.54 ± 0.29 ^{cd}	6.46 ± 0.29 ^b	5.42 ± 0.22 ^b	4.88 ± 0.09 ^b	7.06 ± 0.22 ^{bc}
50 mg/kg bw	14.82 ± 1.37	11.96 ± 0.96 ^{bc}	7.60 ± 0.56 ^b	6.72 ± 0.39 ^b	6.02 ± 0.35 ^b	10.32 ± 0.85 ^b
100 mg/kg bw	15.16 ± 1.01	10.24 ± 0.84 ^{bc}	8.20 ± 0.76 ^b	6.20 ± 0.33 ^b	5.10 ± 0.14 ^b	9.46 ± 0.71 ^b
200 mg/kg bw	16.02 ± 1.94	12.26 ± 1.81 ^{ab}	8.22 ± 1.08 ^b	6.24 ± 0.78 ^b	5.26 ± 0.56 ^b	9.28 ± 1.42 ^b
300 mg/kg bw	14.94 ± 1.38	11.80 ± 0.92 ^{bc}	8.46 ± 0.66 ^b	6.30 ± 0.45 ^b	5.14 ± 0.36 ^b	7.68 ± 0.77 ^{bc}

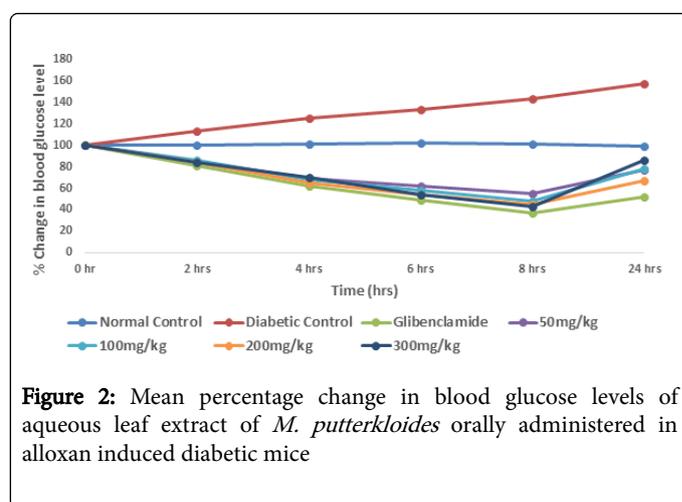
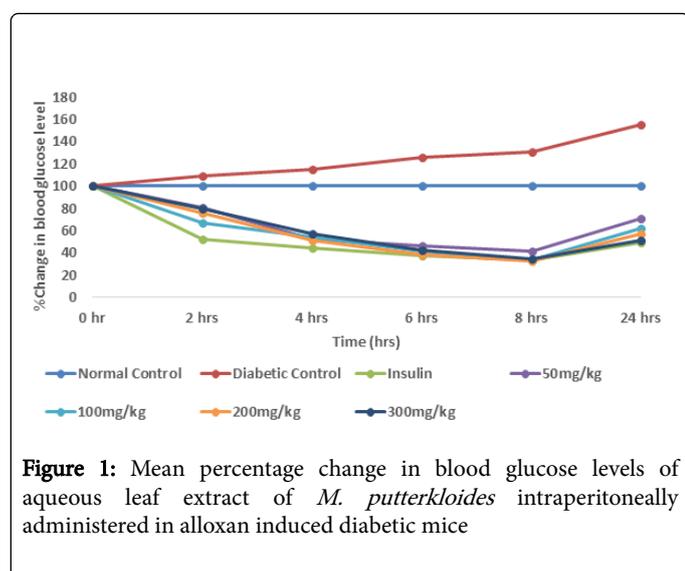
Values are expressed as Mean ± SEM. Values followed by the same superscript are not significantly different (p>0.05; One way ANOVA followed by Tukey's post hoc test)

Table 1: Effects of intraperitoneally administered aqueous leaf extract of *M. putterkloides* on blood glucose levels in alloxan induced diabetic mice (mmol/l).

Treatment Groups	0 hr	2 hr	4 hr	6 hr	8 hr	24 hr
Normal Control	5.12 ± 0.07	5.12 ± 0.04 ^b	5.16 ± 0.02 ^c	5.22 ± 0.02 ^b	5.18 ± 0.04 ^b	5.08 ± 0.04 ^d
Diabetic control	13.76 ± 1.40	15.54 ± 1.69 ^a	17.18 ± 1.59 ^a	18.28 ± 1.50 ^a	19.58 ± 1.69 ^a	21.48 ± 1.56 ^a
Reference group	14.84 ± 1.36	12.26 ± 1.47 ^a	9.36 ± 1.26 ^{bc}	7.28 ± 0.71 ^b	5.30 ± 0.26 ^b	7.48 ± 0.21 ^{cd}
50 mg/kgbw	14.56 ± 1.28	12.20 ± 0.55 ^a	9.66 ± 0.40 ^b	8.66 ± 0.63 ^b	7.70 ± 0.81 ^b	10.96 ± 0.44 ^{bc}
100 mg/kgbw	14.42 ± 0.94	12.50 ± 0.87 ^a	9.86 ± 1.02 ^b	8.46 ± 0.92 ^b	5.50 ± 0.67 ^b	11.26 ± 0.81 ^b
200 mg/kgbw	14.00 ± 1.43	11.80 ± 1.22 ^a	9.06 ± 0.87 ^{bc}	7.38 ± 0.46 ^b	6.20 ± 0.37 ^b	9.16 ± 0.76 ^{bc}
300 mg/kgbw	13.04 ± 0.97	10.94 ± 0.79 ^a	9.06 ± 0.46 ^{bc}	7.00 ± 0.43 ^b	5.50 ± 0.67 ^b	11.24 ± 0.92 ^b

Values are expressed as Mean ± SEM. Values followed by the same superscript are not significantly different (p>0.05; One way ANOVA followed by Tukey's post hoc test)

Table 2: Effects of orally administered aqueous leaf extract of *M. putterkloides* on blood glucose levels in alloxan induced diabetic mice (mmol/l).



Intraperitoneal administration of aqueous leaf extract of the four therapeutic doses (50, 100, 200 and 300 mg/kg body weight) of *M.*

putterkloides also lowered blood glucose levels from the 2nd hour to the 8th hour (Table 1; Figure 1). By the 2nd hour the four therapeutic doses of the extract had lowered the blood glucose levels to 81.12%, 67.43%, 75.52%, and 79.51%, respectively, compared to insulin which had lowered blood sugar levels to 52.10% within the same hour. In the 6th hour the percent blood glucose reductions by the four therapeutic doses of the extract were at 46.13%, 41.26%, 38.96% and 42.60%, respectively, compared to insulin which had lowered blood sugar levels to 37.71% within the same hour. After this, a gradual increase was recorded up to the twenty fourth hour.

Effect of oral and intraperitoneal administration of ethyl acetate leaf extract of *M. putterkloides* on blood glucose levels in alloxan induced diabetic mice

Intraperitoneally administered aqueous leaf extract of *M. putterkloides* decreased the blood glucose levels at all the four doses of 50, 100, 200 and 300 mg/kg body weight (Table 3; Figure 3). The extract caused a steady decline in blood glucose levels from the second to eighth hour. In the 24th hour, there was a gradual increase in blood glucose levels. However, the sugar levels were not reduced in a dose dependent manner.

Treatment Groups	0 hr	2 hr	4 hr	6 hr	8 hr	24 hr
Normal Control	5.16 ± 0.05	5.22 ± 0.04 ^c	5.22 ± 0.04 ^c	5.22 ± 0.02 ^d	5.14 ± 0.02 ^c	5.18 ± 0.05 ^d
Diabetic control	14.26 ± 1.39	16.22 ± 1.54 ^a	17.74 ± 1.71 ^a	19.74 ± 1.61 ^a	21.20 ± 1.57 ^a	22.72 ± 1.34 ^a
Reference group	14.60 ± 1.20	6.68 ± 0.30 ^{bc}	5.96 ± 0.12 ^c	5.52 ± 0.12 ^{cd}	5.14 ± 0.10 ^c	7.020 ± 0.30 ^{cd}
50 mg/kg bw	13.92 ± 1.31	13.22 ± 1.32 ^a	12.00 ± 1.46 ^{ab}	10.86 ± 1.49 ^{bc}	9.50 ± 1.34 ^{bc}	11.58 ± 1.24 ^b
100 mg/kg bw	13.38 ± 1.32	12.44 ± 1.04 ^{ab}	10.96 ± 1.09 ^{bc}	10.24 ± 1.23 ^{bcd}	9.46 ± 1.19 ^{bc}	11.08 ± 0.93 ^{bc}
200 mg/kg bw	15.92 ± 2.03	13.26 ± 1.87 ^a	11.96 ± 1.75 ^{ab}	11.26 ± 1.56 ^b	10.92 ± 1.52 ^b	10.76 ± 1.16 ^{bc}
300 mg/kg bw	15.54 ± 2.29	13.72 ± 1.82 ^a	11.96 ± 1.67 ^{ab}	11.06 ± 1.35 ^b	10.40 ± 1.37 ^{bc}	11.32 ± 1.19 ^{bc}

Values are expressed as Mean ± SEM. Values followed by the same superscript are not significantly different (p>0.05; One way ANOVA followed by Tukey's post hoc test)

Table 3: Effects of intraperitoneally administered ethyl acetate leaf extract of *M. putterkloides* on blood glucose levels in alloxan induced diabetic mice (mmol/l).

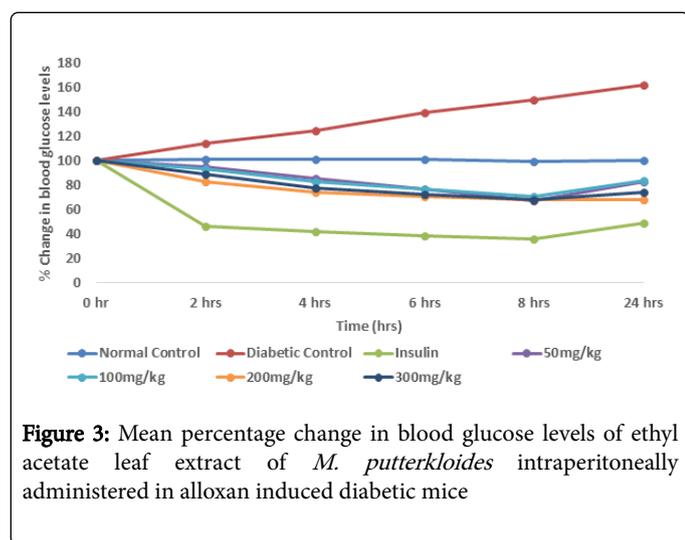


Figure 3: Mean percentage change in blood glucose levels of ethyl acetate leaf extract of *M. putterkloides* intraperitoneally administered in alloxan induced diabetic mice

As depicted in Figure 4 and Table 4, the ethyl acetate leaf extract of *M. putterkloides* at dose levels of 50 and 200 mg/kg body weight demonstrated a significant hyperglycemic activity from the 2nd hour up to the 8th hour while at 100 and 300 mg/kg body weight the extract caused a reduction in blood sugar levels during the same period. By the sixth hour the extract had increased the blood glucose levels to 122.40% and 117.46% (50 and 200 mg/kg respectively) and reduced to 91.51% and 87.63% (100 and 300 mg/kg respectively) for the four doses, compared to 56.77% decrease in blood sugar levels for the conventional oral drug, glibenclamide (Figure 4).

Phytochemicals composition in *M. putterkloides*

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

Discussion

The present study investigated the in vivo hypoglycemic activity of ethyl acetate and aqueous leaf extract of *M. putterkloides* in alloxan-induced diabetic mice. The alloxan destroys and reduces the pancreatic β -cell population via formation of reactive oxygen species like nitric oxide [14]. The observation that both the leaf ethyl acetate and aqueous extracts of *M. putterkloides* in various dosage modes lowered blood glucose indicates that this extract has hypoglycemic constituents.

The possible mechanism through which the extract might have brought about blood glucose lowering effect were either by increasing utilization of glucose or by direct stimulation of glucose uptake through increased insulin secretion [15]. It might also have been due to the extract stimulating β cells in islet of Langerhans, increased serum insulin and reduced blood sugar [16]. Glucose lowering effect might also be attributed to the increased release of insulin from regenerated β -cells [17,18], restored insulin sensitivity [19], interference on absorption of dietary carbohydrates as well as disaccharides in small intestine [20] or facilitated utilization of glucose by peripheral tissues mediated by GLUT-4, an insulin dependent glucose transporter [21]. The lowering of blood glucose levels by *M. putterkloides* in the same manner regardless of the dosage might

suggest that the extract may reflect uptake of the active constituents through saturable active transport; it may also reflect maximum hypoglycemic activity at the lowest dose used (50 mg/kg body weight).

Treatment Groups	0 hr	2 hr	4 hr	6 hr	8 hr	24 hr
Normal Control	5.28 ± 0.07	5.34 ± 0.08 ^b	5.24 ± 0.08 ^c	5.20 ± 0.05 ^d	5.20 ± 0.04 ^c	5.18 ± 0.08 ^d
Diabetic control	14.48 ± 1.74	16.78 ± 1.52 ^a	19.52 ± 1.64 ^a	21.18 ± 1.69 ^a	22.96 ± 1.50 ^a	25.60 ± 1.34 ^a
Reference group	16.18 ± 2.00	13.08 ± 1.38 ^a	8.94 ± 1.06 ^{bc}	6.76 ± 0.51 ^{cd}	5.46 ± 0.27 ^c	7.60 ± 0.36 ^{cd}
50 mg/kgbw	12.80 ± 1.41	13.64 ± 1.67 ^a	14.48 ± 2.39 ^{ab}	15.94 ± 2.91 ^{abc}	18.22 ± 3.88 ^{ab}	17.44 ± 3.31 ^{ab}
100 mg/kgbw	14.28 ± 1.45	14.02 ± 1.41 ^a	13.50 ± 1.63 ^{abc}	13.10 ± 1.81 ^{abcd}	13.10 ± 1.87 ^{abc}	15.62 ± 1.91 ^{bc}
200 mg/kgbw	13.96 ± 1.77	15.76 ± 2.92 ^a	16.24 ± 3.35 ^{ab}	17.36 ± 4.09 ^{ab}	16.62 ± 3.57 ^{ab}	17.10 ± 3.27 ^{ab}
300 mg/kgbw	11.34 ± 1.22	10.46 ± 1.28 ^{ab}	10.22 ± 1.29 ^{bc}	9.92 ± 1.37 ^{bcd}	9.66 ± 1.25 ^{bc}	11.24 ± 1.06 ^{bcd}

Values are expressed as Mean ± SEM. Values followed by the same superscript are not significantly different (p>0.05; One way ANOVA followed by Tukey's post hoc test)

Table 4: Effects of orally administered aqueous leaf extract of *M. putterkloides* on blood glucose levels in alloxan induced diabetic mice (mmol/l).

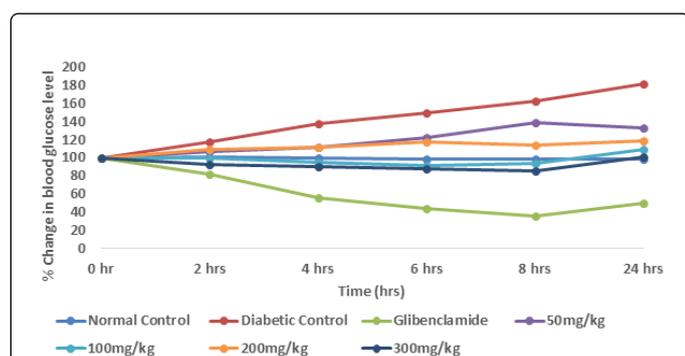


Figure 4: Mean percentage change in blood glucose levels of ethyl acetate leaf extract of *M. putterkloides* orally administered in alloxan induced diabetic mice

Phytochemical constituents of <i>M. putterkloides</i>		
Phytochemicals	Absorbance (nm)	Mean concentration (mg/kg)
Total phenols	650	526.52 ± 0.05
Tannins	700	24.94 ± 0.01
Saponins (%)	-	7.66 ± 0.18
Flavanoids	650	46.40 ± 0.01
Alkaloids (%)	-	5.76 ± 0.15
Vitamin C	-	25.40 ± 4.72
Vitamin B1	-	13.43 ± 1.95
Vitamin B3	-	-

Table 5: Phytochemical composition of *M. putterkloides*

The observation that the two tested doses of ethyl acetate leaf extracts of *M. putterkloides* at 50 and 200 mg/kg body weight dose raised the glucose levels to beyond that of the diabetic control mice suggests that the extract constituents may either have triggered gluconeogenesis together with glycogenolysis or could have had constituents which promoted further death of the pancreas. The observation that both the leaf ethyl acetate (intraperitoneal route) and aqueous extracts (intraperitoneal or oral routes) of *M. putterkloides* in various dosage modes, the sugar levels started rising from the eighth to the twenty fourth hour in all therapeutic dosages may have been due to the extract having a short half-life or the extract may have been prone to fast hepatic metabolism and renal clearance [1].

The hypoglycemic effect of this plant could be associated with the presence total phenolics, saponins, tannins, flavonoids and alkaloids which have been associated with hypoglycemic activity [22]. The alkaloid 1-ephedrine promotes the regeneration of pancreatic islets following destruction of the beta cells, hence restoring the secretion of insulin and thus correcting hyperglycemia [22]. The tannin epigallocatechin-3-gallate exhibits anti-diabetic activity as demonstrated by Broadhurst et al. [23]. Flavonoids isolated from leaf of *Ipomoea batatas*, ameliorated blood glucose level and lipid parameters in alloxan induced diabetic mice at 50-150 mg/kg [24]. Flavonoid fraction from *Pterocarpus marsupium* has been shown to cause pancreatic beta cell regeneration.

Saponins are glycosides of triterpenes, steroids or alkaloids. Previous researchers have demonstrated the hypoglycemic activity of triterpenoid glycosides [25,26]. Presence of saponins in this extract could also be responsible for the hypoglycemic activity. For instance ginseng and its saponins have been shown to lower blood glucose in alloxan-treated, genetically diabetic, and normal mice [26]. Clinically all the forms of tannin may participate in managing glucose level in blood. Tannin stimulates the receptor cells to utilize carbohydrate [27].

The hypoglycemic activity observed could also be due to the presence of various macronutrients such as vitamin B1 and B3 and vitamin C which are strong antioxidants and have been known to manage diabetes mellitus. They do so by neutralizing the reactive oxygen species (hydroxyl and superoxide radicals, hydrogen peroxide,

lipid peroxides) generated during the induction of diabetes by alloxan by helping the pancreatic cells to regenerate and hence helping to maintain optimal cellular and systemic health and well-being of the mice [28]. These antioxidants interact and synergistically stabilize, or deactivate, free radicals before they attack cells [29]. Antioxidants are responsible for most of the biological activities of phytochemicals including antidiabetic, anti-inflammatory, antiallergenic, antiviral, anti-aging and anti-carcinogenic activities [30,31].

Conclusion

In conclusion, the findings of this study indicate that, oral administration of ethyl acetate leaf extract of *M. putterkloides* at doses of 50 and 200 mg/kg body weight, respectively demonstrated hypoglycemic activity while 100 and 300 mg/kg lowered blood sugar levels in a dose independent. The aqueous leaves demonstrated hypoglycemic activity in a dose independent in all therapeutic dose levels in both routes. The observed hypoglycemic activity could be associated with the phytochemicals and phytonutrients present in *M. putterkloides*. The study recommends continued use of *M. putterkloides* in the management of diabetes and support the folkloric claim. Also the study finding opens an avenue for the conservation to ensure that its continued use is sustainable.

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