

In Vivo Antidiabetic Activity of the Aqueous Leaf Extract of *Croton macrostachyus* in Alloxan Induced Diabetic Mice

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Abstract

The folklore reports from traditional medical practitioners that *Croton macrostachyus* has bioactivity against several diseases including diabetes mellitus have not been scientifically evaluated. The aim of this study was to determine the *in vivo* hypoglycemic activity of aqueous leaf extracts of this plant in male swiss white albino mice. Aqueous leaf extract of *Croton macrostachyus* was intraperitoneally and orally administered to alloxan (180.9 mg/kg; intraperitoneally)-induced diabetic mice at different doses of 25 mg/kgbw, 48.4 mg/kgbw, 93.5 mg/kgbw, 180.9 mg/kgbw and 350 mg/kgbw and the effects on blood glucose levels investigated. The treatments effects were compared with three controls (normal, diabetic and diabetic treated with a standard antidiabetic drugs (insulin administered intraperitoneally at 1 IU/kg body weight in 0.1 ml physiological saline or glibenclamide administered orally at 3 mg/kg body weight in 0.1 ml physiological saline). Phytochemical composition of the leaf extracts were qualitatively assessed using standard procedures. The diabetic control mice showed significantly ($p < 0.05$) higher fasting blood glucose when compared with normal control mice. Treatment of diabetic mice with doses of the leaf extract resulted in significantly ($p < 0.05$) lower levels of fasting blood glucose. The effects of the leaf extract were comparable with the conventional drugs. However, the glucose lowering potency of this plant extract was dose independent. The aqueous leaf extracts contained tannins, flavonoids, saponins, sterols, anthraquinones and alkaloids. The observed hypoglycemic activity could be associated with the phytochemicals present in this plant extract. Therefore, the results suggest that *Croton macrostachyus* leaf extract is a potent hypoglycemic agent and this validates their folkloric usage. Further studies to investigate the mechanism of action for hypoglycemic activity for these plant species should be done in order to explore possibilities of developing a drug that can function by similar mode of action as the plant extract.

Keywords: Diabetes mellitus; *Croton macrostachyus*; Hypoglycemic activity; Antidiabetic; Phytochemicals

Introduction

Diabetes mellitus is a metabolic disorder characterized by high blood sugar levels that results from either an inherited or acquired deficiency in the production of insulin by the pancreatic islet cells of Langerhans or by the ineffectiveness of the insulin produced at the peripheral tissues [1]. Diabetes mellitus is a chronic medical condition which though can be controlled lasts a lifetime [2]. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels [3]. An estimated 171 million people were suffering from diabetes in 2000, and this number could total 366 million by 2030 [4]. In 2012, 1.5 million deaths were reported to be directly caused by diabetes [5].

Diabetes mellitus can be categorized into type I or insulin-dependent diabetes mellitus (IDDM) also referred to as juvenile onset diabetes resulting from a cellular mediated autoimmune destruction of β -cells in the pancreas [6]. It accounts for 5-10% globally of individuals with diabetes. The other category is type II or non-insulin-dependent diabetes mellitus (NIDDM) whose onset is usually after 40 years of age [6] and accounts for approximately 90-95% of the diabetes mellitus cases world-wide. It is also called adults onset diabetes which affects individuals who have insulin resistance and usually have relative insulin deficiency [6].

The clinical manifestation of diabetes mellitus include excessive excretion of urine (polyuria), thirst (polydipsia), constant hunger (polyphagia), weight loss, blurred vision and fatigue [5]. Acute, life

threatening consequences of uncontrolled diabetes are hyperglycemia with ketoacidosis or the non-ketotic hyperosmolar syndrome [7]. Long term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction [7].

Normal fasting plasma glucose levels ranges between 3.5-6.7 mmol/l (63-120.6 mg/dl). After a carbohydrate 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 (2000 mg/L) is considered to be diagnostic criteria for diabetes and correlates with Hb A1C threshold of 6.5% [8].

The mainstay of non-pharmacological treatment of diabetes is

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diet and physical activity [9]. However, other methods of treatment such as acupuncture and hydrotherapy, mineral supplementation, conventional drugs which include exogenous insulin and oral hypoglycemic agents and transplantation [9] has been bedevilled by prohibitive costs, need for expertise in prescription and administration and numerous side effects, which are precursors of complications [4]. Therefore, herbal prescriptions have received considerable attention as an alternative way to compensate for perceived deficiencies in orthodox pharmacotherapy [4].

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 [10]. 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 [11]. The chemical composition of herbal products and potency depends on the plant extract derivative, the age of the plant part used, season when harvested and the methods of processing [12].

Croton macrostachyus has been in use by traditional health practitioners to treat different human diseases. Boiled leaf decoction of *Croton macrostachyus* is drunk or ashes taken orally as treatment for cough; juice from fresh leaves is applied on wounds to hasten clotting [13]. Root decoction is used as an anthelmintic for tapeworm, as a purgative, and for malaria and venereal diseases. Bark from the stems and roots is boiled in water and newly born babies are bathed in the mixture as a remedy for skin rash [13]. The plant has also been in use successfully in the management of diabetes mellitus in some parts of Kenya. However, its increased use has not been accompanied by an increase in the quantity, quality and accessibility of clinical evidence to support traditional medicine practitioner's claims. This study therefore contributes additional knowledge to the use of *Croton macrostachyus* in the management of diabetes from samples collected from Nyamira County, Kenya.

Materials and Method

Study site

This study was undertaken at the Department of Biochemistry and Biotechnology, School of Pure and Applied Sciences, Kenyatta University from July 2013 to February 2015. Kenyatta University is 23 km from Nairobi off Thika Road.

Collection and preparation of the plant materials

The plants used in this study were collected from their native habitats on the basis of ethnobotanical information. They were collected with bioconservation aspects in mind from Kijauri village Nyamira county Kenya. Information on the identity of the plant to collect, the precise locality where it grows, what part to collect, when curative potency is at maximum and the mode of preparation was provided by a traditional medical practitioner. For this study, the parts of the plants collected were the leaves. Botanical identities of the plants were authenticated by an acknowledged authority in taxonomy and a voucher specimen deposited at the National Museums of Kenya Herbarium, Nairobi.

Leaves were collected while green and dried at room temperature away from direct sunlight for different periods of time depending on their succulence. The dried leaves were separately ground into fine powder by use of an electric mill. The powdered plant materials were kept at room temperature away from direct sunlight in closed, dry plastic air tight bags ready for extraction.

Preparation of the aqueous extracts

Each one hundred grams of the powdered plant material was extracted in 1 liter distilled water at 60°C for 6 hour. 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 hour. The freeze-dried powder was then weighed and stored in airtight container at -20°C until used for bioassay.

Experimental animals

The study used male Swiss White Albino mice (3-4 weeks old) that weighed 21-25 g with a mean weight of 23 g. These were bred in the Animal house at the Department of Biochemistry and Biotechnology of Kenyatta University. The mice were housed at a temperature of 25°C with 12 hours/12 hours darkness photoperiod and fed on rodent pellets and water *ad libitum*. The experimental protocols and procedures used in this study were approved by the Ethics Committee for the Care and Use of Laboratory Animals of Kenyatta University, Kenya.

Induction of hyperglycemia

Hyperglycemia was induced experimentally by a single 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) [14].

Forty-eight hours after alloxan administration, blood glucose level was measured using a glucometer. Mice with blood glucose levels above 200 mg/dL were considered diabetic and used in this study. Prior to initiation of this experiment, the animals were fasted for 8-12 hours [15] 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 eight 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 25 mg/kg body weight in 0.1 ml physiological saline; Group V consisted of alloxan induced diabetic mice either intraperitoneally or orally administered with 48.4 mg/kg body weight in 0.1 ml physiological saline; Group VI consisted of alloxan induced diabetic mice either intraperitoneally or orally administered with 93.5 mg/kg body weight in 0.1 ml physiological saline; Group VII consisted of alloxan induced diabetic mice either intraperitoneally or orally administered with 180.9 mg/kg body weight in 1 ml physiological saline. Group VIII consisted of alloxan induced diabetic mice either intraperitoneally or orally administered with 350 mg/kg body weight in 1 ml physiological saline. 0.1ml 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 1, 2, 3, 4, 6 and 24 hours. Bleeding was enhanced by gently “milking” the tail from the body towards the tip. After the operation, the tips of the tail were sterilized by swabbing with 70% ethanol. The blood glucose levels were determined with a glucose analyser model (Hypogaurd, Woodbridge, England).

Qualitative phytochemical screening

A phytochemical screening of alkaloids, flavonoids, saponins, tannins, terpenoids, sterols, and free and bound anthraquinones present in *Croton macrostachyus* extracts was performed using standard methods [16,17].

Data management and statistical analysis

The Data was entered in the Microsoft Excel Spread Sheet, cleaned and then exported to SAS statistical software version 9.1.3 for analysis. Results were expressed as Mean \pm Standard Deviation (SD) of the number of animals used per every study point. Statistical analysis were done using ANOVA and post-ANOVA to compare the means of untreated normal control mice with diabetic mice treated with saline, diabetic mice treated with the conventional drug, and diabetic mice treated with plant extract at doses of 25 mg/kg body weight, 48.4 mg/kg body weight, 93.5 mg/kg body weight, 180.9 mg/kg body weight and 350 mg/kg body weight. The values of $p \leq 0.05$ were considered to be significant.

Results

Effect of oral and intraperitoneal administration of aqueous leaf extracts of *Croton macrostachyus* on blood glucose levels in alloxan induced diabetic mice

The aqueous leaf extracts of *Croton macrostachyus* yielded a 7% light brown powder. Upon intraperitoneal administration, the aqueous leaf extracts of *Croton macrostachyus* decreased the blood glucose levels at all the five doses of 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight (Table 1). This occurred in three phases, whereby in the first hour, the extract caused a steep decline in blood glucose levels, followed by a steady decline from the second to seventh hour. A gradual increase was then observed in the twenty fourth hour. However, the sugar levels were not reduced in a dose dependent manner. In the first hour, the extracts lowered blood glucose levels to 82.1%, 90.3%, 55.3%, 75.4% and 71.2% for 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight doses, respectively, compared to insulin treated diabetic mice whose blood sugar levels were lowered to 50.2% within the first hour. By the third hour, all the five doses had lowered blood sugar levels to 44.1%, 46.4%, 41.5%, 49.7% and 44.0% respectively, compared to insulin treated diabetic mice whose sugar levels were lowered to 31.1% within the same hour (Figure 1).

Upon oral administration, the aqueous leaf extracts of *Croton macrostachyus* also lowered blood glucose levels at all the five doses of 25, 48.4, 93.5, 180.9 and 350 mg/kg body weight from the first hour to the seventh hour in a dose-independent manner. A gradual increase was then observed from the seventh to the twenty fourth hours. However, by the second hour, the extract had lowered the blood glucose levels to 71.7%, 68.0%, 58.9%, 58.3% and 69.8% respectively for the five doses, compared to 50.2% for the conventional oral drug, glibenclamide (Figure 2). This is a dose-independent response. The reduction in blood glucose levels when compared to the negative control was statistically significant ($P \leq 0.05$).

TREATMENT	BLOOD GLUCOSE LEVELS AT VARYING TIMES (mmoles/L)						
	0 hr	1 hr	2 hr	3 hr	4 hr	7 hr	24 hr
Control/ Saline	5.20 \pm 0.07 ^c	5.34 \pm 0.11 ^e	5.32 \pm 0.08 ^d	5.22 \pm 0.08 ^b	5.16 \pm 0.05 ^b	5.22 \pm 0.08 ^b	5.24 \pm 0.15 ^c
Diabetic/ Saline	14.52 \pm 2.97 ^{ba}	16.02 \pm 2.48 ^a	17.52 \pm 2.62 ^a	19.20 \pm 2.88 ^a	21.22 \pm 3.23 ^a	22.50 \pm 3.48 ^a	25.00 \pm 2.98 ^a
Diabetic/ insulin	17.46 \pm 2.99 ^a	8.62 \pm 0.55 ^{edc}	6.20 \pm 0.56 ^{cd}	9.32 \pm 0.29 ^b	4.96 \pm 0.11 ^b	4.70 \pm 0.28 ^b	6.30 \pm 0.79 ^{cb}
25 (mg/kgbw)	15.42 \pm 1.26 ^{ba}	12.68 \pm 3.11 ^{bac}	8.90 \pm 1.64 ^{cb}	6.80 \pm 1.06 ^b	5.44 \pm 0.78 ^b	4.68 \pm 0.48 ^b	6.64 \pm 1.15 ^{cb}
48.4 (mg/kgbw)	16.48 \pm 2.91 ^{ba}	14.98 \pm 3.49 ^{ba}	10.46 \pm 2.36 ^b	7.60 \pm 1.25 ^b	6.24 \pm 1.23 ^b	5.18 \pm 0.74 ^b	8.48 \pm 1.44 ^b
93.5 (mg/kgbw)	13.68 \pm 2.55 ^{ba}	7.48 \pm 0.91 ^{ed}	6.30 \pm 0.46 ^{cd}	5.54 \pm 0.43 ^b	5.90 \pm 0.72 ^b	4.62 \pm 0.36 ^b	8.12 \pm 1.54 ^{cb}
180.9 (mg/kgbw)	12.50 \pm 1.02 ^b	9.42 \pm 1.04 ^{adc}	7.28 \pm 0.98 ^{abd}	6.16 \pm 0.23 ^b	5.48 \pm 0.34 ^b	5.02 \pm 0.15 ^b	7.84 \pm 0.59 ^{cb}
350 (mg/kgbw)	15.96 \pm 3.33 ^{ba}	11.36 \pm 2.76 ^{adc}	8.88 \pm 2.08 ^{cb}	6.94 \pm 1.30 ^b	5.68 \pm 0.98 ^b	5.14 \pm 0.70 ^b	6.64 \pm 0.55 ^{cb}

Results are expressed as Means \pm SD for five mice per group. Values followed by the same superscript are not statistically different ($P \leq 0.05$; analysed by ANOVA followed by Tukey's post hoc test).

Table 1: Effects of intraperitoneally administered aqueous leaf extracts of *Croton macrostachyus* on blood glucose levels in alloxan induced diabetic mice

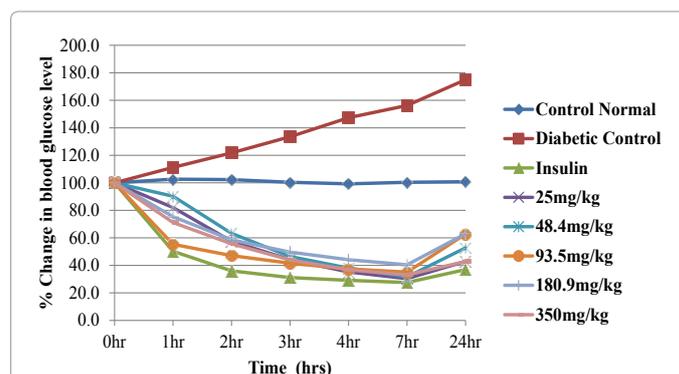


Figure 1: Mean percentage change in blood glucose levels of *Croton macrostachyus* intraperitoneally administered in alloxan induced diabetic mice.

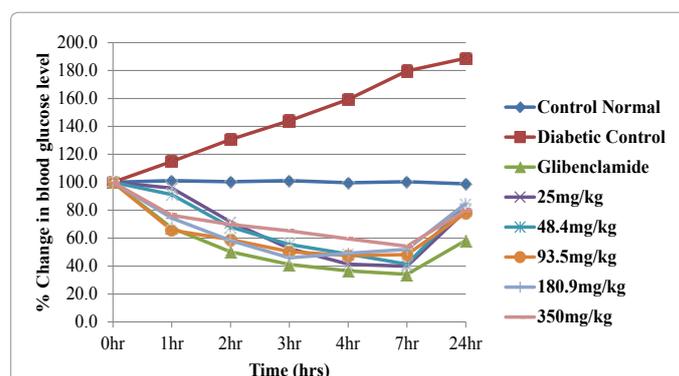


Figure 2: Mean percentage change in blood glucose levels of *Croton macrostachyus* orally administered in alloxan induced diabetic mice.

Qualitative analysis of the phytochemical composition of aqueous leaf extracts of *Croton macrostachyus*

The phytochemical screening of the aqueous leaf extracts of *Croton macrostachyus* indicated the presence of Alkaloids, Saponins, Terpenoids, Flavonoids, Tannins Free and Bound Athraquinones as shown in Table 2.

Discussion

This study was carried out to investigate the *in vivo* antidiabetic effect of the aqueous leaf extracts of *Croton macrostachyus* in alloxan induced diabetic mice and normal mice respectively. Alloxan destroys and reduces the β -cells via formation of reactive oxygen species like nitric oxide [15]. The alloxan-induced diabetic mice had 3 to 4 times increase in blood glucose levels compared to normal control group. Both oral and intraperitoneal administration of the aqueous extract of the studied plant showed hypoglycemic activity at the five tested dose levels (25 mg/kg body weight, 48.4 mg/kg body weight, 93.5 mg/kg body weight, 180.9 mg/kg body weight, and 350 mg/kg body weight) in a dose independent manner.

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 [9].

The hypoglycemic activity of some plants have been identified and experimentally demonstrated in the *in-vivo* and *in-vitro* diabetic models and documented in several studies. These plants include: *Azadirachta indica* [18,19], *Momordica charantia* [20], *Cola acuminata* [21] and *Phyllanthus amarus* [22].

The mode of action of the extract may have been by insulinomimeticism [4] as is the mode of action of some oral hypoglycemic drugs such as biguanides, which relieve hyperglycemia by lowering hepatic gluconeogenesis, up regulating skeletal muscle glucose uptake, and limiting plasma triacylglycerols [23].

The blood glucose ameliorative effect of the aqueous leaf extract of *Croton macrostachyus* could be attributed to the presence of heterogeneous phytoconstituents such as alkaloids, saponins, tannins, terpenoids, flavanoids, anthraquinones and sterols that have been associated with hypoglycemic activity [24] (Table 3). The presence of flavonoids, sterols and saponins has previously been reported in ethanolic fruit extracts of *L. camara* Linn which demonstrated hypoglycemic activity in streptozotocin induced diabetic male wistar rats [9].

TREATMENT	BLOOD GLUCOSE LEVELS AT VARYING TIMES (mmoles/L)						
	0 hr	1 hr	2 hr	3 hr	4 hr	7 hr	24 hr
Control/ Saline	5.16 ± 0.11 ^b	5.22 ± 0.08 ^c	5.18 ± 0.08 ^b	5.22 ± 0.08 ^{cb}	5.14 ± 0.09 ^b	5.18 ± 0.08 ^b	5.10 ± 0.10 ^c
Diabetic/ Saline	14.92 ± 4.99 ^a	16.98 ± 5.25 ^a	18.80 ± 4.63 ^a	20.50 ± 4.01 ^a	22.44 ± 3.41 ^a	24.88 ± 2.41 ^a	26.14 ± 2.27 ^a
Diabetic/Glen	14.66 ± 3.03 ^a	9.82 ± 2.06 ^{bc}	7.38 ± 1.88 ^b	5.96 ± 0.93 ^{cb}	5.28 ± 0.79 ^b	4.80 ± 0.37 ^b	8.42 ± 1.93 ^b
25 (mg/kgbw)	12.96 ± 3.59 ^a	12.44 ± 4.84 ^{ba}	9.14 ± 2.37 ^b	6.52 ± 1.45 ^{cb}	5.02 ± 0.88 ^b	4.80 ± 0.86 ^b	10.04 ± 1.62 ^b
48.4 (mg/kgbw)	10.64 ± 2.57 ^{ba}	9.72 ± 5.36 ^{bc}	7.12 ± 1.99 ^b	5.76 ± 1.36 ^{cb}	5.08 ± 1.38 ^b	4.44 ± 1.67 ^b	8.88 ± 2.08 ^b
93.5 (mg/kgbw)	12.42 ± 1.18 ^a	8.18 ± 1.11 ^{bc}	7.32 ± 0.75 ^b	6.24 ± 0.65 ^{cb}	4.98 ± 0.25 ^b	5.98 ± 1.35 ^b	9.64 ± 1.17 ^b
180.9 (mg/kgbw)	9.14 ± 1.11 ^{ba}	6.78 ± 1.70 ^{bc}	5.32 ± 1.19 ^b	4.20 ± 1.11 ^c	4.44 ± 0.90 ^b	4.68 ± 0.83 ^b	7.76 ± 1.23 ^{cb}
350 (mg/kgbw)	12.72 ± 3.47 ^a	9.66 ± 2.74 ^{bc}	8.74 ± 1.95 ^b	8.18 ± 2.03 ^b	7.94 ± 1.26 ^b	6.62 ± 0.69 ^b	9.90 ± 1.27 ^b

Table 2: Effects of orally administered aqueous leaf extracts of *Croton macrostachyus* on blood glucose levels in alloxan induced diabetic mice.

Phytochemicals	<i>Croton macrostachyus</i>
Alkaloids	+
Sterols	+
Terpenoids	+
Saponins	+
Tannins	+
Flavanoids	+
Free and Bound Anthraquinones	+

Key: Present phytochemicals are denoted by (+) sign, absent phytochemicals are denoted by (-) sign

Table 3: Qualitative phytochemical screening of aqueous leaf extract of *Croton macrostachyus*.

Flavonoids act on various molecular targets and regulate different signaling pathways in pancreatic β -cells, hepatocytes, adipocytes and skeletal myofibers [2]. Oral administration of the flavonoids content (8%) of the seeds of *Cuminum nigrum* caused a significant blood glucose lowering at a dose range of 0.5 to 1.5 g/kg, both in normoglycaemic and alloxan-induced diabetic rabbits [25]. Flavonoid and terpenoids isolated from the other antidiabetic medicinal plants has been found to stimulate insulin secretion or possess an insulin like-effect [26]. Effect of the flavonoids quercetin and ferulic acid on pancreatic β -cells leads to their proliferation and secretion of more insulin have been proposed by [27,28] as the mechanism by which they reduced hyperglycaemia caused by streptozocin in diabetic rats. Flavonoid fraction from *Pterocarpus marsupium* has been shown to cause pancreatic beta cell regranulation. Epicatechin, its active principle, has been found to be insulinogenic thus enhancing insulin release and conversion of proinsulin to insulin *in vitro* [29].

Saponin fraction isolated from *Memordica charantia* reduced blood glucose levels and increased insulin secretion and glycogen synthesis in alloxan induced diabetic mice [30]. As reported by [31] ginseng and its saponins have lowered blood glucose in alloxan-treated, genetically diabetic, and normal mice. Total saponins from the seeds of *Entada phaseoloides* significantly decreased fasted blood glucose and attenuated hyperglycemia associated oxidative stress in type 2 diabetic rats [32].

The presence of alkaloids in this extract could also be responsible for the hypoglycemic 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 [9]. Intraperitoneally administered alkaloids isolated from leaves of *Acanthus montanus* at doses of 100, 200 and 400 mg/kg body weight showed hypoglycemic action in alloxan-induced diabetic rats [33]. Alkaloids from *Ephedra distachya* herbs induced increased insulin secretion by causing regeneration and restoration of atrophied pancreatic islets [34].

Due to the presence of terpenoids, the leaves and seeds of *E. officinalis* are used in the treatment of diabetes [35]. *L. rhodesiensis* extracts contain anthraquinones which have previously been reported to lower blood glucose [36]. Reported that *Polygonum multiflorum* extracts containing anthrax-quinones 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 [37,38].

Commercially available tannic acids induced phosphorylation of the insulin receptor (IR) and act, as well as translocation of glucose transporter 4 (GLUT 4), the protein factors involved in the signaling

pathway of insulin-mediated glucose transport [39,40]. Reported that all the forms of tannins may participate in managing glucose level in blood. Tannin stimulates the receptor cells to utilize carbohydrate. The presence of tannins in *R. tridentata* might have brought about blood glucose lowering effect. Condensed tannins extracted from some kenyan foods showed antihyperglycemic action due to inhibition of α -amylase and α -glucosidase enzymes [41].

Conclusion

The aqueous leaf extracts of *Croton macrostachyus* had antidiabetic activity. The intraperitoneal route was more effective than the oral route as assessed from the rate of glucose reduction and its half-life. Further, qualitative phytochemical screening of aqueous leaf extracts of *Croton macrostachyus* indicated the presence of phenols, alkaloids, flavonoids, tannins, saponins, sterols and free and bound anthraquinones. The antidiabetic effect of the studied plant may have resulted from its phytochemical constituents. This study, therefore, recommends use of aqueous stem extracts of *C. macrostachyus* in management of diabetes mellitus. Moreover, further studies on the use of organic extracts will be necessary as it would also reveal classes of organic secondary metabolites in this plant that may be hypoglycemic.

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