Phytopharmacology

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Antidiabetic activity of the root extract of *Detarium microcarpum* (Fabacaee) Guill and Perr.

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Received: 10 March 2012, **Revised**: 26 March 2012, **Accepted**: 26 March 2012

Abstract

Diabetes mellitus is a common endocrine disorder that impairs glucose homeostasis resulting in severe diabetic complications including retinopathy, angiopathy, nephropathy, and neuropathy thus causing neurological disorder. In this study, antidiabetic activity of root extract of *Detarium microcapum* was investigated in rat model of diabetes. A methanol root extract was prepared by soxhlet extraction and was separated into fraction using chloroform, n-hexane and methanol to yield chloroform fraction (CF), n-hexane fraction (HF) and methanol fraction (MF). The extract and its fractions were screened for phytochemicals using standard methods. The acute toxicity (LD_{50}) of the extract was determined in mice. Diabetes was induced by a single ip injection of 120 mg/kg of alloxan monohydrate and glucose level was analyzed as indices of diabetes. The acute toxicity test showed that the root bark extract was safe at doses of up to 5 g/kg. The phytochemical screening of the plant revealed the presence of proteins, carbohydrates and terpenoids in large amount while saponins, resins, glycosides and flavonoids were present in moderate amount. The results indicated that intraperitoneal injection of ME, MF, CF and HF reversed the effect of alloxan in rats by different degrees. The antidiabetic potency of the extract and fractions was in the order MF > ME > HF > CF. The results of this study justify the use of this plant roots as traditional treatment for diabetes mellitus.
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Keywords: Diabetes mellitus; *Detarium microcarpum*, blood sugar, alloxan

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder resulting from insulin deficiency, characterized by hyperglycemia, altered metabolism of carbohydrates, proteins and lipids, and an increased risk of vascular complication (Barar, 2004). DM develops due to a diminished insulin production (Type 1) or resistance to its effect (Type 2) (WHO, 1999).

Conventionally, type I diabetes is managed with exogenous insulin and type 2 with oral hypoglycemic agents (sulphonylureas, biguanides etc). In traditional practice, medicinal plants are used in many countries to control diabetes mellitus. DM has recently been identified by Indian Council of Medical Research (ICMR) as one of the refractory diseases for which satisfactory treatment is not available in modern allopathic system of medicine and suitable herbal preparations are to be investigated. (Verma et al, 2010).

Detarium microcarpum Guill and Perr (Fabaceae) is a small tree up to 10m tall; the root system is horizontal. It is confined to Africa. It is typically a species of the dry savanna and occurs in humid forest (Leung et al, 1968). Different parts of this plant have been reported to possess antirheumatic activity (Abreu et al, 1998) and antiplasmodial activities (Kouyate, 2005). The plant is also used against malaria, leprosy and impotence (Baerts and Lehmann, 2002). The seeds and leaves are eaten as a condiment and vegetable (Njoku et al, 1999). A wide range of chemical constituents have been isolated from this plant viz diterpen-es (Witting and Guinko 1998), water soluble polysacharides proteins and coumarins (Neuwinger, 1996). In view of acclaimed antidiabetic potential of *D. microcarpum*, the blood sugar lowering activity of the plant was investigated in normal and diabetic rats.

Material and methods

Plant material

The roots of *D. microcapum* were collected from Orba area in Nsukka District, Enugu State, Nigeria and anthenticated by Mr. A.O. Ozioko of Bioresources Development and Conservation Programme (BDCP) Laboratory, Nsukka, Enugu State Nigeria. A voucher specimen (No. AO/BDCP/264) is deposited in the Department of Pharmacognosy, University of Nigeria, Nsukka. The dried root was subjected to size reduction to a coarse powder by using local dry grinder and passed through sieve. About 500 g of this powder was packed into soxhlet apparatus and extracted exhaustively with methanol to obtain methanol extract (24.4 w/w) and the extract was subsequently separated into fractions using methanol, chloroform and n-hexane in increasing order of polarity to yield MF, CF and HF respective-ely. The solvent was recovered by distillation *in racuo* and extracts were stored in dessicator and used for subsequent assays.

Phytochemical screening

The extract and fractions from *D. microcarpum* were subjected to various qualitative test for the identification of various plant constituents. (Harborne 1998).

Animals

Healthy adult male and female albino rats between 2 and 3 months of age and weighing between 150-200 g were used for the study. They were housed in Animal House of Department of Pharmacology and Toxicology University of Nigeria, Nsukka. Enugu State, Nigeria. They were fed with standard rat pellet diet (Topfeeds PLC, Nigeria). All animal experiments were conducted in accordance to the NIH guide for the care and use of laboratory

animals, and approved by the University Ethical Committee for the use of laboratory animals.

Acute toxicity study (LD_{50})

The oral acute toxicity of the methanol extract (ME) was determined in mice as described by Lorke (1983). Briefly, nine mice randomly divided into three groups (n=3) were orally administered 10, 100 and 1000 mg/kg of ME respectively, and observed for 24 hours for death. When no death occurred, 1600, 2900 and 5000 mg/kg of ME was administered to a fresh batch of animals (n=1) and the number of death in 24 hours noted. The LD_{50} is estimated as the geometric mean of the highest nonlethal dose and the lowest lethal dose (Lorke, 1983).

Induction of diabetes

Diabetes was induced by intraperitoneal injection of 120 mg/kg of alloxan monohydrate. (Kannur et al, 2006). The alloxanized rats were kept for 7 days with free access food and water. On the 8^{th}, the rats were fasted for 12 hours and their blood sugar levels were determined using Accu-chek Active glucometer (USA). Rats with glucose levels above 120 mg/dl were used for the study.

Treatment protocol

The non-diabetic rats were randomly divided into five group (n = 6/group). Group 1 received 2 ml/kg of normal saline while group 2 received 2 mg/kg glibenclamide. Group 3, 4 and 5 received 500, 1000, 2000 mg/kg of methanol extract respectively. Same procedures were performed using diabetic rats at same doses of methanol extract. In another series of experiment MF, CF and HF (1000 mg/kg) were administered to diabetic rats (n = 6). Blood sample were collected from the tail vein after overnight fast at the internal of 0, 2, 4, 8, 16, 32 hours of treatment (Akah et al, 2011). The blood glucose levels were estimated using Accu–chek Active™ glucose strips in Accu-Chek Active™ Test glucometer (USA).

Statistical analysis

Data obtained were analyzed using one way Analysis of variance (ANOVA) (SPSS version 14) software and expressed as mean ± SEM. Differences between means were regarded significant at $P < 0.05$ (LSD post hoc test).

Results

Preliminary phytochemical screening

Preliminary phytochemical screening of the extract and fractions revealed the presence of large amount of proteins, carbohydrates and terpenoids while flavonoids, saponins, glycosides and resins were moderately present as shown in Table 1.

Acute toxicity profile

No death was recorded up to doses of 5000 mg/kg, indicating the relative safety of the extract.

Table 1. Result of Preliminary Phytochemical screening

Phytochemicals	ME	MF	CF	HF
Saponins	+ +	+ +	-	+
Proteins	+ + +	+ + +	-	-
Tannins	-	-	-	-
Carbohydrates	+ +	+ +	-	-
Reducing Sugar	+	+	-	-
Resins	+ +	-	+ +	+
Flavonoids	+ +	+	+ +	-
Alkaloids	-	-	-	-
Glycosides	+ +	+ +	-	-
Terpenoids	+ + +	+ + +	-	-
Steroids	+ + +	-	+	+ +
Fats and Oil	+ +	-	+	+ +
Acidic Compounds	-	-	-	-

- = Not present, + = Present in small concentration, ++ = Present in moderate concentration, +++ = Present in high concentration.

Antihyperglycemic activity of methanol extract on normal and alloxan diabetic rats

The effects of methanol extract of *D. microcarpum* on normal and diabetic rats are shown in Fig 1 and 2. In normal rats, no significant ($p > 0.05$) reduction in blood sugar was observed at the 3 doses administered. However, administration of 2000 mg/kg of the extract to diabetic rats caused a marked significant ($p < 0.05$) reduction in the fasting blood sugar level in all the hours. With other doses (500 and 1000 mg/kg), the reduction became significant ($P<0.05$) from 8th to 32nd hours.

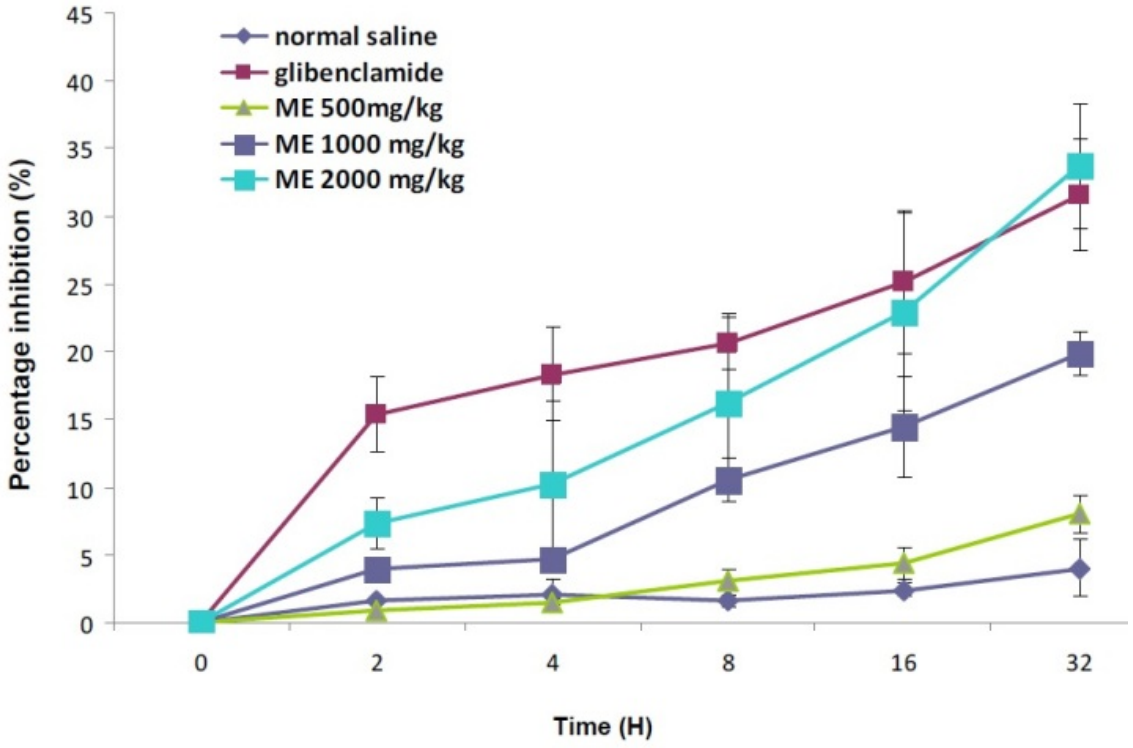

Figure 1. Effect of methanol extract on blood glucose level in normal rats

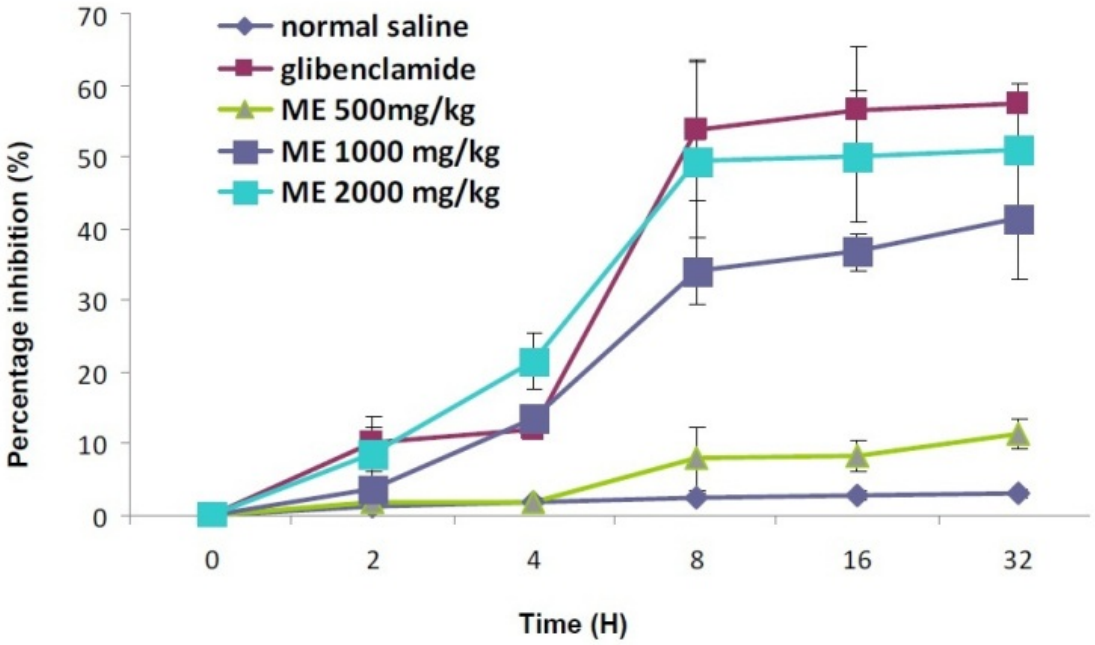

Figure 2. Effect of methanol extract on blood glucose level in diabetic rats

Antihyperglyacemic activity of the fractions on diabetic rats

The methanol fraction and glibenclamide caused significant ($P < 0.05$) reduction in the blood glucose level of diabetic rats from the 2nd hour up to 32nd hour of treatment (67.3% and 57.5% at the 32nd hours respectively). The chloroform fraction caused significant ($p < 0.05$) reductions at 16th hour and 32nd hour while the n-hexane fraction had no significant effect (Figure 3).

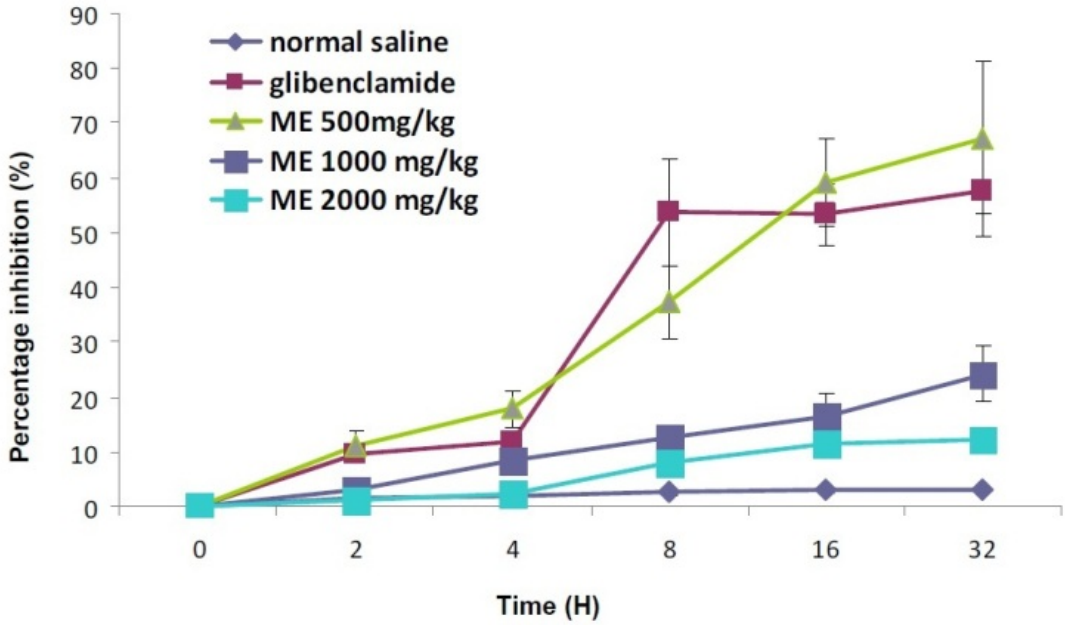

Figure 3. Effect of fractions on blood glucose level in diabetic rats

Discussion

Diabetes mellitus is a major degenerative disease in the world today affecting at least 15 million people and having complications which include hypertension, atherosclerosis and microcirculatory disorders. There have been increasing demand for the use of plant products for the management of diabetes due to low cost, easy availability and lesser side effect (Sherma et al, 2010). Therefore, in this study we investigated the use of *D. microcarpum* as antidiabetic agent.

Alloxan was used to induce diabetes because it is known to cause damage to the beta cells of the pancreas (Lenzen, 2008). It is now established that there is a gradual decrease in beta-cell function and mass that may occur in individual at high risk of developing type 2 diabetes. To prevent the loss of beta-cell function and mass, beta- cell stabilization and regeneration must occur (Chakravarthy et al, 1982).

The result of this present study indicated that *D. microcarpum* root extract and fraction reduced significantly the blood sugar level in alloxan diabetic rats. The methanol extract exhibited significant ($p < 0.05$) antihyperglycemic effect without causing hypoglycaemia (Figure 1). The methanol fraction produced similar effect like glibenclamide. Glibenclamide directly improves insulin action and is effective only in the presence of insulin (Bailey, 1992). It is not evident whether the antidiabetic effect of *D. microcarpum* may be due to increased insulin secretion as occurred with glibenclamide. The antidiabetic effect of the extract may be partly due to the presence of flavonoids. It is reported that flavonoids constit-ute the active biological principles of most medicinal plants with hypoglycaemic and antidia-betic properties (Wollenweber et al, 1988).

In conclusion, the results of this study revealed that the root extract of *D. microcarpum* exhibited significant antihyperglycemic activity, and this justify its popular use in traditional management of diabetes.

Conflict of interest

There is no conflict of interest associated with the authors of this paper.

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