Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Medicine

journal homepage:www.elsevier.com/locate/apjtm

Document heading doi:

Evaluation of the anti-diabetic properties of *Mucuna pruriens* seed extract

Stephen O Majekodunmi[1,2], Ademola A Oyagbemi[3], Solomon Umukoro[4], Oluwatoyin A Odeku [2*]

[1]*Department of Pharmaceutics and Pharmaceutical Technology, Faculty of Pharmacy, University of Uyo, Uyo, Nigeria*
[2]*Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria*
[3]*Department of Veterinary Physiology, Biochemistry & Pharmacology, University of Ibadan, Nigeria*
[4]*Department of Pharmacology and Therapeutics, College of Medicine, University of Ibadan, Ibadan, Nigeria*

ARTICLE INFO

Article history:
Received 8 April 2011
Received in revised form 11 May 2011
Accepted 15 June 2011
Available online 20 August 2011

Keywords:
Antidiabetic
Mucuna pruriens
Hypoglycemic activity
Toxicity
Diabetes mellitus

ABSTRACT

Objective: To explore the antidiabetic properties of *Mucuna pruriens*(*M. pruriens*). **Methods:** Diabetes was induced in Wistar rats by single intravenous injection of 120 mg/kg of alloxan monohydrate and different doses of the extract were administered to diabetic rats. The blood glucose level was determined using a glucometer and results were compared with normal and untreated diabetic rats. The acute toxicity was also determined in albino mice. **Results:** Results showed that the administration of 5, 10, 20, 30, 40, 50, and 100 mg/kg of the crude ethanolic extract of *M. pruriens* seeds to alloxan-induced diabetic rats (plasma glucose > 450 mg/dL) resulted in 18.6%, 24.9%, 30.8%, 41.4%, 49.7%, 53.1% and 55.4% reduction, respectively in blood glucose level of the diabetic rats after 8h of treatment while the administration of glibenclamide (5 mg/kg/day) resulted in 59.7% reduction. Chronic administration of the extract resulted in a significant dose dependent reduction in the blood glucose level ($P<0.001$). It also showed that the antidiabetic activity of *M. pruriens* seeds resides in the methanolic and ethanolic fractions of the extract. Acute toxicity studies indicated that the extract was relatively safe at low doses, although some adverse reactions were observed at higher doses (8-32 mg/kg body weight), no death was recorded. Furthermore, oral administration of *M. pruriens* seed extract also significantly reduced the weight loss associated with diabetes. **Conclusions:** The study clearly supports the traditional use of *M. pruriens* for the treatment of diabetes and indicates that the plant could be a good source of potent antidiabetic drug.

1. Introduction

Diabetes mellitus is a growing threat to public health in modern society[1]. It is a metabolic disorder of the pancreas in which blood sugar (glucose) levels are abnormally high (hyperglycaemia) because either the body does not produce enough insulin, the hormone produced by beta cells of the islet of Langerhans that controls the amount of sugar in the blood[2], or the insulin produced cannot be used by the body[3]. While some non insulin dependent diabetic mellitus patients can be managed by diet alone, others require hypoglycaemic therapy and/or insulin. Although insulin therapy affords an effective glycaemic control, drawbacks such as oral ineffectiveness, short shelf life, requirement of constant refrigeration, parenteral therapy with its attendant abscesses and fatal hypoglycaemia in the event of excess dosage limit its usage. On the other hand, pharmacotherapy with sulphonylureas, biguanides and thiazolidones is also associated with side effects[4]. Therefore, there is an urgent need to find safe and effective pharmacological interventions for diabetes mellitus.

In recent years, the popularity of complementary medicine has increased considerably and the WHO has suggested the evaluation of potentials of plants as effective therapeutic agents, especially in areas where modern drugs are not readily available[4]. Due to their relative safety and low costs, herbal medicinal plants are prescribed even when they are not standardized[5]. One of the known antidiabetic medicinal plants used worldwide is *Mucuna pruriens* L (Fam. Leguminoseae) (*M. pruriens*) also known as velvet beans or Cowitch in English. The local names in Nigeria are: *Werepe* in Yoruba; *Karara* in Hausa and *Agbara* in Ibo. It is an annual climbing plant indigenous to tropical regions, especially Africa, India and the West Indies where it is found in bushes, bush paths and hedges. Its flowers are white to dark purple and hang in clusters. The plant also produces clusters of pods, which contains seeds known

*Corresponding author: Oluwatoyin A Odeku, Department of Pharmaceutics and Industrial Pharmacy, Faculty of Pharmacy, University of Ibadan, Ibadan, Nigeria.
E-mail: pejuodeku@yahoo.com, o.odeku@mail.ui.edu.ng

as Mucuna beans. Seed pods are covered with reddish-orange hair-like needles that are readily dislodged and can cause intense irritation to the skin. These hairs contain mucunain and serotonin and causes itching, blisters and dermatitis[6]. The seed is a natural source of L-dopa (about 7%-10%) and they are also rich in novel alkaloids, saponin and sterols[6]. Because of its high L-dopa content it is used in the management of Parkinson's disease and neurological illnesses[7]. Various species of *Mucuna* are grown as a minor food crop in Ghana, Mozambique, and Nigeria and is still eaten as food during famine and specialty food in northeastern India. Raw *Mucuna* bean seeds are rich in minerals especially K, Mg, Ca, and Fe, and contain about 27% protein[7].

Seeds of *M. pruriens* have been shown to possess antispasmodic, anti-inflammatory, antipyretic and antivenin properties[8-10], aphrodisiac activity[11], and anabolic and fertility properties[10]. *M. pruriens* has been reported to be useful in India, Brazil, Spain and Germany, in the treatment of diabetes[12]. Although the hypoglycaemic effect of the alcohol extract of seeds of *M. pruriens* has been demonstrated in normal[13] and streptozotocin[12] and alloxan[13] induced diabetic rats, the anti-hyperglycaemic effect was observed only at high doses (200-400 mg/kg/day) after 3 weeks and the maximum effect obtained after 15 week of daily administration of 200 mg/kg/day was 47%. Moreover, effects of the administration of the extract on body weight and the acute toxicity of the extract have remained largely uninvestigated. Thus, in the present study, the antidiabetic properties and acute toxicity of various concentrations of the ethanolic seed extract and fractions of *M. pruriens* has been evaluated in normal and alloxan-induced diabetic rats and compared with standard hypoglycaemic drug, glibenclamide. The ability of *M. pruriens* extract to reduce body weight loss in the diabetic rats has also been evaluated.

2. Materials and methods

2.1. Chemicals

Alloxan monohydrate was obtained from Sigma Chemical Co. (St. Louis, M.O., USA) and a glibenclamide tablet was obtained from the Nigerian-German Chemicals PLC, (Otta, Ogun State, Nigeria.). All reagents were of analytical grade.

2.2. Collection of plant materials and extracts preparation

Seeds of *M. pruriens* were collected at Tose village, Ibadan, Nigeria and authenticated by the Herbarium Unit of the Department of Botany and Microbiology, University of Ibadan, Ibadan, Nigeria. A voucher specimen has been kept in the herbarium of the Department (Voucher No. UIH 22305). Seeds of *M. pruriens* were dried under shade then powdered with a milling machine and then sieved (particle size of 180 μm). To prepare the crude ethanolic extract, 100 g of the ground seed was extracted with 500 mL of ethanol at room temperature. The extract was dried and then powdered and sieved (particle size 0.18 μm). A yield of 44% w/w was obtained.

To prepare the fractions, 10 g of ground seed was also successively extracted in chloroform, *n*-hexane, ethylacetate, methanol and ethanol, and dried as described for the crude ethanolic extract. A yield of 0.540 g, 0.827 g, 0.679 g, 1.006 g and 1.009 g was obtained for chloroform, *n*-hexane, ethylacetate, methanol and ethanol, respectively.

2.3. Animals

Wistar rats (180-240 g) and albino mice (16-20 g) of both sexes were obtained and bred in the animal house of the Department of Veterinary Pharmacology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria. After randomization into various groups, animals were acclimatized for a period of 2-3 days before the start of experiments. Animals described as fasting had been deprived of food for at least 16 h but had been allowed free access to drinking water.

2.4. Induction of diabetes

Diabetes was induced by single intravenous injection of 120 mg/kg of alloxan monohydrate (dissolved just before use in 0.9% normal saline) to overnight fasted rats[14]. Animals, in which the development of hyperglycaemia was confirmed 72 h after the administration of alloxan injection (blood glucose level range of 450-500 mg/dL) were used for experiments.

2.5. Acute hypoglycaemic activity

Sixty Wistar rats were randomly allocated into ten groups (A-J) with six rats in each treatment. Group A consisted of non treated rats (control), Group B consisted of diabetic control rats (alloxan induced diabetic rats), Groups C, D, E, F, G, H and I were diabetic rats and 5, 10, 20, 30, 40, 50 and 100 mg/kg body weight, respectively of the crude ethanolic seed extract were administered orally while diabetic rats in group J received glibenclamide (5 mg/kg) as standard reference drug. Blood glucose levels were measured at zero time (before receiving the extract) and at various time intervals for 24 h.

2.6. Chronic hypoglycaemic activity

For the chronic study, sixty Wistar rats were randomly allocated into ten groups (A-J) with six rats in each group. Animals in each group were administered with the same dose of extracts and standard drug daily as described for acute studies. The blood glucose level was measured weekly for 12 weeks. The animals were carefully monitored and weighed every week.

2.7. Effect of fractions of M. pruriens on diabetic rats

Thirty diabetic Wistar rats were randomly allocated into five groups with six rats in each treatment. Each group received 10 mg/kg of the *n*-hexane, chloroform, ethylacetate, methanol and ethanol fractions, respectively and the blood glucose level was measured at different time intervals using a glucometer (LifeScan Inc. California, USA).

2.8. Acute toxicity studies

In the acute toxicity study, thirty six albino mice were

distributed into six groups consisting of 6 rats each. Mice were given 2, 4, 6, 8, 16 and 32 g/kg body weight, respectively of the ethanol extract of *M. pruriens*. Doses for the extract were selected based on constant logarithm ratio of 2. Mice were deprived of food overnight prior to the oral administration of the extract and were observed for toxic signs, symptoms and mortality for two weeks.

2.9. Statistical analysis

Data were expressed as mean±SD. Statistical analysis were performed using the analysis of variance (ANOVA) followed by Tukey post test. Differences were considered to be significant when $P < 0.05$ unless otherwise stated.

3. Results

Results of the effect of the crude ethanolic seed extract of *M. pruriens* on the blood glucose level in alloxan-induced diabetic rats are presented in Table 1. It showed that the administration of 5, 10, 20, 30, 40, 50, and 100 mg/kg of the crude ethanolic extract of *M. pruriens* seed resulted in 18.6%, 24.9%, 30.8%, 41.4%, 49.7%, 53.1% and 55.4% reduction, respectively in blood glucose of the diabetic rats after 8 h of treatment while the administration of glibenclamide (5 mg/kg body weight) resulted in 59.7% reduction. There was a significant reduction in the blood glucose level of the rats compared with the diabetic standard ($P<0.0001$). However, there were no statistically significantdifferences in the % reduction of the blood glucose level when the extract was administered at a dose of ⩾50mg/kg/day and the standard drug, glibenclamide ($P>0.05$).

Result of the effect of chronic administration of *M. pruriens* extracts on the blood glucose level is presented in Table 2 while the plots of % reduction in the blood glucose level versus time (weeks) after chronic administration of different concentrations of the extract and standard drug to diabetic rats are presented in Figure 1. It indicated that daily administration of the extract of *M. pruriens* seed for 12 weeks resulted in a dose-dependent reduction in the blood glucose level. Daily administration of 5, 10, 20, 30, 40, 50 and 100 mg/kg of the extract resulted in 55.2%, 59.5%, 68.4%, 74.7%, 80.9%, 83.2% and 83.6% reduction in blood glucose level, respectively after 12 weeks. On the other hand, the standard drug, glibenclamide resulted in 57.8% reduction in the plasma glucose levels of the diabetic rats.

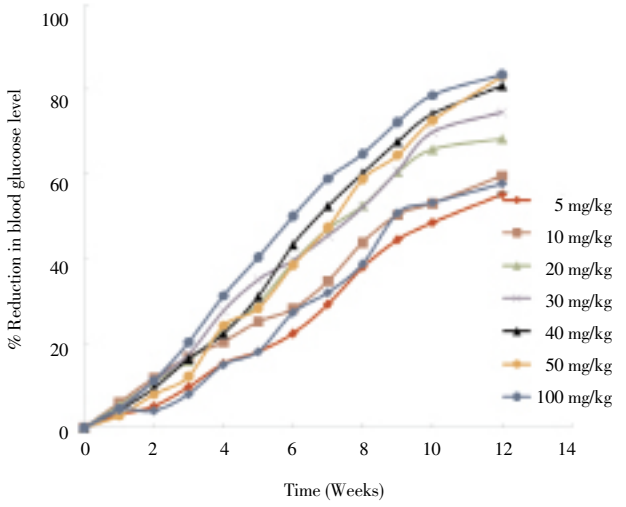

Figure 1. Plots of % reduction in the blood glucose level versus time (weeks) after chronic administration of different concentrations of the extract and standard drug to diabetic rats.

To determine the activity of fraction of the extract, the powdered seed of was subjected to successive extraction with *n*-hexane, chloroform, ethyl acetate, methanol and ethanol and the hypoglycaemic effect of these fractions is presented in Table 3. It showed that *n*-hexane, chloroform and ethyl acetate fractions possessed no hypoglycaemic activity at the dose employed. However, methanol and ethanol fractions showed 39.1% and 38.9% reduction in the blood glucose level, respectively, 24 h after the administration of extracts.

Acute toxicity studies showed that the administration of the crude ethanolic extract of *M. pruriens* seed was well tolerated by animals as no death was recorded 14 days after the administration of the extract. However, adverse effects such as hyperventilation, clonic seizures, pico erection and reduced spontaneous motor activity were observed about 1 hour after the administration of high doses (8-32 g/kg/day).

Changes in body weights of diabetic rats after daily administration of the crude ethanolic extract of *M. pruriens* seed are shown in Table 4. It indicated that the normal

Table 1

Effect of ethanolic extract of *M. pruriens* seeds on the blood glucose level of alloxan-induced diabetic rats (mg/dL) (Mean ± SD, $n = 6$).

Time (Hour)	Group A (Normal control)	Group B (Diabetic control)	Group C (5 mg/kg)	Group D (10 mg/kg)	Group E (20 mg/kg)	Group F (30 mg/kg)	Group G (40 mg/kg)	Group H (50 mg/kg)	Group I (100 mg/kg)	Group J (Glibenclamide)
0	74.4 ± 6.3	455.3 ± 1.9$^{\triangle}$	455.8 ± 4.7*	460.4± 2.3*	469.5± 3.2*	486.7 ± 8.6*	475.6 ± 6.4*	470.7 ± 5.6*	452.6 ± 5.5*	470.5±3.7*
1	75.6 ± 8.2	457.6 ± 1.4$^{\triangle}$	450.4 ± 3.5*	456.4 ± 5.3*	462.4 ±9.7*	472.8 ± 5.8*	444.8 ± 5.4*	460.4 ± 5.9*	401.0 ± 3.5*	386.4 ± 3.5*
2	75.7 ± 6.9	459.5 ± 2.9$^{\triangle}$	450.5 ± 4.7*	448.7 ± 4.5*	440.0 ±5.6*	435.5 ± 3.6*	426.7 ± 4.2*	430.8 ± 3.1*	381.8 ± 3.6*	386.4 ± 3.5*
3	69.9 ± 7.4	461.6 ± 1.5$^{\triangle}$	440.5 ± 2.7*	426.4 ± 5.2*	427.7 ±1.7*	420.7 ± 2.3*	402.5 ± 5.7*	396.7 ± 4.6*	345.5 ± 2.7*	364.8 ± 3.5*
4	71.3 ± 8.4	463.8 ± 1.4$^{\triangle}$	432.7 ± 7.6*	414.5 ± 3.8*	402.6 ±4.8*	400.7 ± 6.8*	370.5 ± 4.6*	363.0 ± 6.5*	368.5 ± 9.7*	281.7 ± 6.4**
5	71.4 ± 7.3	465.6 ± 2.5$^{\triangle}$	423.7 ± 5.6*	400.9 ± 4.5*	375.0 ±6.9*	377.6 ± 4.7*	345.7 ± 4.5**	328.9 ± 4.5**	324.8 ± 4.1**	271.7 ± 1.8**
6	77.4 ± 7.9	466.9 ± 1.9$^{\triangle}$	407.8 ± 4.7*	385.7 ± 3.6*	357.2 ±7.1**	337.6 ± 9.5**	310.2 ± 7.3**	282.8 ± 2.6**	261.7 ± 3.9**	241.7 ± 9.1**
7	73.9 ±10.7	468.4 ± 1.1$^{\triangle}$	385.5 ± 1.5*	365.5 ± 5.1**	342.4 ±3.1**	308.7 ± 6.4**	275.9 ± 7.6**	255.8 ± 4.2**	241.4 ± 2.7**	210.8 ±8.6**
8	70.9 ±11.6	470.6 ± 1.1$^{\triangle}$	370.9 ± 2.6*	345.7 ± 2.1**	325.0 ± 2.4**	285.2 ± 7.2**	239.2 ± 8.6**	220.6 ± 4.9**	201.7 ± 3.7**	189.7 ± 5.6**
24	76.7 ±9.8	474.3 ± 4.1$^{\triangle}$	351.2 ± 1.8*	327.3 ± 3.2**	300.0 ± 3.2**	254.7 ± 5.4**	202.5 ± 6.7**	185.5 ± 4.2**	171.2 ± 2.8**	165.8 ± 2.4**

$^{\triangle}$: $P<0.05$, *vs.* Group A; *: $P<0.05$, **: $P<0.01$, *vs.* Group B.

Table 2
Effect of chronic administration of ethanol extract of *M. pruriens* seeds on blood glucose level of alloxan-induced diabetic rats (mg/dL) (mean ± SD, $n = 6$).

Time Week)	Group A (Normal control)	Group B (Diabetic control)	Group C (5 mg/kg)	Group D (10 mg/kg)	Group E (20 mg/kg)	Group F (30 mg/kg)	Group G (40 mg/kg)	Group H (50 mg/kg)	Group I (100 mg/kg)	Group J (Glibenclamide)
1	77.5 ± 0.5	461.9 ± 7.4	464.5 ± 5.4	464.2 ± 4.3	454.8 ± 5.3	470.7 ± 4.7	459.8 ± 6.7	467.8 ± 8.6	470.6 ± 9.7	470.3 ± 7.1
2	75.9 ± 0.6	468.6 ± 6.4	450.5 ± 2.4	436.6 ± 3.6	430.6 ± 2.1	448.6 ± 5.7	440.9 ± 4.7	454.5 ± 5.4	449.8 ± 2.4	450.1± 4.0
3	78.6 ± 0.6	474.5 ± 4.5	440.7 ± 3.8	409.5 ± 7.5	405.9 ± 3.4	418.5 ± 6.8	416.8 ± 5.8	430.9 ± 1.4	418.2 ± 8.9	451.2 ± 8.9
4	78.5 ± 0.2	480.6 ± 5.7	419.6 ± 6.5	386.6 ± 4.6	382.9 ± 4.1	388.8 ± 8.5	384.7 ± 6.8	410.7 ± 2.4	375.4 ± 3.4	432.9 ± 7.4
5	74.8 ± 1.4	487.4 ± 6.3	393.8 ± 4.8	369.8 ± 4.3	350.3 ± 6.9	340.4 ± 7.5	357.5 ± 4.8	354.9 ± 3.6	323.7 ± 7.1	400.1 ± 3.5
6	76.8 ±1.2	495.0 ± 3.2	380.5 ± 3.5	347.7 ± 5.7	320.9 ± 5.8	305.7 ± 4.8	316.9 ± 6.4	335.4 ± 7.6	280.8 ± 2.7	385.5 ± 4.8
7	77.9 ± 2.4	502.2 ± 4.6	360.7 ± 3.2	333.7 ± 4.3	276.7 ± 4.8	284.2 ± 8.3	260.6 ± 7.4	287.7 ± 1.4	235.2 ± 4.1	342.1 ± 5.3
8	78.3 ± 3.2	509.4 ± 6.4	328.7 ± 6.3	303.4 ± 5.4	240.7 ± 9.6	256.7 ± 8.5	218.9 ± 4.3	246.2 ± 2.3	193.4 ± 5.4	319.8 ± 7.8
9	78.6 ± 1.3	517.8 ± 5.7	287.9 ± 8.5	260.8 ± 4.9	215.8 ± 4.9	224.9 ± 9.6	182.9 ± 5.6	192.4 ± 0.1	165.6 ± 7.1	287.7 ± 6.6
10	75.8 ± 1.2	525.8 ± 4.3	257.8 ± 7.3	230.6 ± 7.5	179.9 ± 1.5	184.7 ± 5.3	148.8 ± 4.8	165.9 ± 1.4	130.4 ± 4.6	231.4 ± 4.5
11	76.5 ± 1.3	535.9 ± 6.5	238.8 ± 7.5	217.8 ± 4.4	155.3 ± 1.4	141.4 ± 7.4	118.5 ± 6.5	127.6 ± 4.4	100.4 ± 4.6	219.7 ± 2.4
12	78.7 ± 1.4	545.6 ± 6.3	207.7 ± 4.8	187.8 ± 5.9	143.8 ± 0.6	119.2 ± 3.1	87.9 ± 6.2	78.7 ± 2.4	77.4 ± 3.7	198.7 ± 6.2

Values are statistically significant at $P < 0.001$ when alloxan treated was compared with the normal control and experimental groups.

Table 3
Effect of crude fractions of *M. pruriens* seeds on blood glucose level on alloxan-induced diabetic rats (mg/dL) (mean ± SD, $n = 6$).

Time (hour)	n-hexane	Chloroform	Ethylacetate	Methanol	Ethanol
0	455.5 ± 0.4	456.2 ± 0.7	456.8 ± 0.5	466.7 ± 0.3	460.1 ± 0.2
1	455.5 ± 1.2	457.1 ± 1.4	457.2 ± 0.9	447.7 ± 0.2	451.5 ± 2.3
2	456.6 ± 2.4	457.6 ± 1.3	457.7 ± 0.3	438.3 ± 2.7	440.2 ± 1.0
3	456.4 ± 0.8	456.4 ± 1.3	458.7 ± 2.1	430.4 ± 0.3	433.9 ± 3.4
4	457.2 ± 0.4	457.2 ± 1.3	458.6 ± 1.6	387.2 ± 0.7	382.1 ± 0.5
5	457.3 ± 1.4	457.3 ± 0.5	459.6 ± 4.6	387.5 ± 2.4	382.6 ± 2.6
6	456.1 ± 0.7	458.6 ± 3.7	460.3 ± 7.8	362.7 ± 1.5	337.7 ± 5.7
7	458.8 ± 0.4	458.4 ± 2.7	460.4 ± 6.3	324.6 ± 4.2	337.7 ± 3.5
8	458.5 ± 0.3	459.2 ± 1.3	459.6 ± 7.3	300.6 ± 3.6	305.2 ± 3.8
24	464.5 ± 1.6	463.7 ± 0.6	460.5 ± 1.2	284.3 ± 4.3	281.3 ± 2.6

Table 4
Effect of chronic administration of ethanol extract of *M. pruriens* seeds on the body weight of diabetic rats (mean ± SD, $n = 6$).

Time (Week)	Group A (Normal control)	Group B (Diabetic control)	Group C (5 mg/kg)	Group D (10 mg/kg)	Group E (20 mg/kg)	Group F (30 mg/kg)	Group G (40 mg/kg)	Group H (50 mg/kg)	Group I (100 mg/kg)	Group J (Glibenclamide)
0	163.2±11.5	225.0±7.9	192.0±10.5	220.0±12.5	195.0±14.6	190.0±10.0	226.5±16.4	235.0±9.9	237.2±3.2	193.0±14.5
1	165.3±13.6	223.5±14.7	193.2±11.7	224.2±13.2	198.8±12.7	195.7±14.7	231.4±13.6	237.2±11.7	238.5±4.5	194.2±1.5
2	168.3±10.5	220.2±8.9	197.4±13.7	228.3±9.4	205.8±8.9	220.6±7.6	237.3±11.6	240.0±14.5	241.3±6.3	196.2±9.9
3	170.2±11.6	218.9±9.9	199.3±12.5	231.9±11.6	210.8±11.3	227.6±11.5	242.4±8.9	242.3±12.4	244.2±10.3	198.3±9.5
4	175.2±7.9	214.0±11.8	2033±12.5	234.8±14.6	214.2±13.6	234.7±9.7	256.4±13.7	244.3±13.7	246.4±9.4	201.2±14.5
5	178.2±11.8	211.4±14.8	208.3±11.5	237.0±11.2	217.4±9.7	238.5±11.5	260.3±11.3	246.3±11.8	250.2±10.2	204.1±11.4
6	183.1±10.7	202.8±13.5	214.2±13.8	242.8±14.6	224.4±14.5	243.7±14.7	266.4±12.8	2492±14.6	254.2±8.6	206.2±12.4
7	187.4±13.6	189.8±9.9	218.0±9.6	246.5±14.7	229.4±12.9	246.9±9.9	271.3±13.5	2541±13.5	256.5±7.5	211.2±14.3
8	193.9±11.3	182.0±11.8	223.2±10.7	249.0±12.6	236.3±14.1	252.5±13.5	275.2±9.7	258.2±9.7	258.2±8.9	216.3±9.4
9	197.3±14.7	177.6±12.8	226.2±11.7	255.8±9.6	239.3±14.4	257.7±12.7	279.2±13.5	265.0±14.5	260.2±9.6	219.4±13.8
10	201.2±13.6	171.5±13.7	230.4±12.8	258.2±10.5	246.8±12.8	261.2±8.9	283.4±14.6	269.0±13.3	263.2±10.3	223.5±14.3
12	207.8±14.9	165.0±11.8	234.0±14.5	263.3±15.6	251.8±11.8	266.4±15.5	286.3±12.8	273.2±12.5	265.4±9.7	227.2±13.5

control showed 27% weight gain while diabetic control rats exhibited 27% weight loss over the 12 weeks period. However, rats that were administered with the extracts exhibited 16%-40% weight gain while those administered with glibenclamide (5 mg/kg) showed 18% weight gain. The weight gain observed with the extract depended on the dose administered. This indicates that treatment with *M. pruriens* seed extract significantly alleviated the weight loss in diabetic rats in a similar manner to glibenclamide ($P<0.001$).

4. Discussion

Beside drugs classically used for the treatment of diabetes, several species of plants have been described as having a hypoglycaemic activity[14-16]. These herbal medicines have

been recommended for the treatment of diabetes and are considered less toxic with fewer side effects than synthetic ones[15].

The administration of single dose of the ethanolic extract of *M. pruriens* resulted in a significant reduction in the blood glucose level of the diabetic rats when compared with diabetic rats that received no treatment(P<0.001). The hypoglycaemic effect was found to be dose dependent with the administration of 5, 10, 20, 30, 40, 50 and 100 mg/kg of the extract to diabetic rats resulting in 22.9%, 28.9%, 36.1%, 47.7%, 57.4%, 60.6% and 62.2% reduction in blood glucose, respectively, 24 hours after the administration while glibenclamide resulted in 64.8% reduction in the blood glucose level. Antidiabetic effects of extracts at high doses (50 mg/kg/day and above) is comparable with effects of glibenclamide (5 mg/kg).

The extract exhibited a significant dose dependent reduction in the blood glucose level after chronic administration(P<0.001). The maximum hypoglycaemic effect (83.6% reduction) was achieved by the extract at a dose of 100 mg/kg. Furthermore, daily administration of 5 mg/kg of the extract for 12 weeks resulted in 55.3% reduction in blood glucose level which is similar to the effect achieved by glibenclamide (5 mg/kg/day). There was a significant difference between effects of chronic administration of the extract at high doses (50 mg/kg and above) and those of glibenclamide (5 mg/kg) (P<0.001). Thus, the extract appears to have a stronger ability to reduce the blood glucose level after long term use than glibenclamide. The hypoglycaemic effect obtained in this study is significantly higher than those reported by Rathi *et al*[12], who obtained a maximum hypoglycaemic effect of 47.7% after 15 weeks of daily administration of 200 mg/kg/day of the alcohol extract of *M. pruriens* obtained in India. Variations due to weather conditions, maturity of the plant and soil composition significantly affect the pharmacological properties of herbs and therefore the actual dose of active ingredient[17]. The hypoglycaemic effect of the *M. pruriens* seed was found to reside in methanol and ethanolic fractions. *n*-hexane, chloroform and ethyl acetate fractions did not have any antidiabetic effects.

Diabetes is often associated with the characteristic loss of body weight which is partially due to increased muscle wasting[18]. Treatment of diabetic rats with the extract was also led to a reduction in the weight loss usually associated with diabetes. The rats exhibited a significant dose dependent weight gain similar to those achieved by the normal rats and those receiving the standard drug, glibenclamide(P<0.001).

The study indicates that the ethanolic seed extract of *M. pruriens* possess antidiabetic activities comparable with the standard drug, glibenclamide. Acute toxicity studies showed that the extract was relatively safe except at high doses when some adverse effects were observed although no death was recorded. Furthermore, oral administration of *M. pruriens* seed extract also reduced the weight loss associated with diabetes. The study clearly supports the traditional use of the plant for the treatment of diabetes.

Conflict of interest statement

We declare that we have no conflict of interest.

References

[1] Xiang L, Huang X, Chen L, Rao P, Ke L. The reparative effects of *Momordica charantia* Linn extract on HiT-T15 pancreatic beta cells. *Asia Pac J Clin Nutrit* 2007; **16**: 249-252.

[2] Rother KI. Diabetes treatment: Bridging the divide. *New Engl J Med* 2007; **356**(15): 1499-1501.

[3] Mayfield J. Diagnosis and classification of diabetes mellitus- New Criteria. *J Amer Acad Fam Physic* 1998; **58**: 11-15.

[4] Swanston-Flatt SK, Day C, Bailey CJ, Flatt PR. Traditional plant treatments for diabetes: studies in normal and streptozotocin diabetic mice. *Diabetologia* 1990; **33**: 462-464.

[5] Majekodunmi SO, Adegoke OA, Odeku OA. Formulation of the extract of the stem bark of *Alstonia boonei* as tablet dosage form. *Trop J Pharm Res* 2008; **7** (2): 987-994.

[6] Katzenschlager R, Evans A, Manson A, Patsalos PN, Ratnaraj N, Watt H, et al. *Mucuna pruriens* in Parkinson's disease: a double blind clinical and pharmacological study. *J Neurosurg Psych* 2004; **75**: 1672-1677.

[7] Olaboro G. Amino acid, mineral trypsin inhibitor and proximate analyses of velvet beans and bean fractions. *Discov Innov* 1993; **5**(2): 163-166.

[8] Josephine RM, Janardhanan K. Studies on chemical composition and antinutritional factors in three germplasm seed materials of the tribal pulse, *Mucuna pruriens* (L.) DC. *Food Chem* 1992; **43**: 13-18.

[9] Guerranti R, Aguiyi JC, Leoncini R, Pagani R, Cinci G, Marinello E. Characterization of the factor responsible for the antisnake activity of *Mucuna pruriens* seeds. *J Prev Med Hyg* 1999; **1**: 25-28.

[10] Guerranti R, Aguiyi JC, Leoncini R, Pagani R, Cinci G, Marinello E. Protein from *Mucuna pruriens* and enzymes from *Echis carinatus* venon- Characterization and cross-reaction. *J Biol Chem* 2002; **277**: 17072-17078.

[11] Dhawan BN, Dubey MP, Mehrortra BN, Rastogi RP, Tandon JS. Screening of Indian plants for biological activity. Part IX. *Indian J Exp Biol* 1980; **18**: 594-606.

[12] Rathi SS, Grover JK, Vats V. The effect of *Momordica charantia* and *Mucuna pruriens* in experimental diabetes and their effect on key metabolic enzymes involved in carbohydrate metabolism. *Phytother Res* 2002; **16**: 236-243.

[13] Silva FRMB, Szpoganiez B, Pizzolatti MG, Willrich MA, De Sousa E. Acute effect of *Bouhinia forficate* on serum glucose levels of normal and alloxan induced diabetic rats. *J Ethnopharmacol* 2002; **83**: 33-37.

[14] Verspohl EJ. Recommended testing in diabetes research. *J Planta Med* 2002; **68:** 581-590.

[15] De Sousa E, Zanatta L, Seifriz I, Creczynski-Pasa TB, Pizzolatti MG, Sypoganicz B, Silva FRMB. Hypoglycemic effect and antioxidant potential of kaempferol-3,7-0-(α)-dirhamnoside from Bauhinia forficate leaves. *J Nat Prod* 2004; **67**: 829-832.

[16] Colca JR. Insulin sensitizers may prevent metabolic inflammation. *J Biochem Pharmacol* 2006; **72**: 125-131.

[17] Alarcon-Aguilar FJ, Jimenez-Estrada M, Reyes-Chilpa R, Roman-Ramos R. Hypoglycemic effect of extracts and fractions from *Psacalium decompositum* in healthy and alloxan diabetic mice. *J. Ethnopharmacol* 2000; **72**: 21-27.

[18] Lau A, Holmes MJ, Woo S and Koh H. Analysis of adulterants in a traditional herbal medicinal product using liquid chromatography-mass spectroscopy. *J Pharm Bio Ana* 2003; **31**: 401-406.