ANTI-HYPERGLYCEMIC EFFICIENCY OF THE AQUEOUS SEED EXTRACT OF *MUCUNA PRURIENS* IN NICOTINAMIDE-STREPTOZOTOCIN-INDUCED DIABETIC *WISTAR* RATS.

Abstract

This study was undertaken to evaluate the remedial effect of the aqueous seed extract of *Mucuna pruriens* (ASEMP) on the endocrine region of pancreas of nicotinamide-streptozotocin-induced diabetes in *Wistar* rats. The anti-hyperglycemic efficiency of two varieties of *Mucuna pruriens var utilis*; IIHR Selection 3 and Arka Dhanvantari was investigated. Oral administration of ASEMP against the nicotinamide-streptozotocin-induced diabetes in the *Wistar* rats showed anti-hyperglycemic effect on the blood glucose level (94 ± 0.2 mg/dl ASEMP 400 mg/kg) when compared with the control group (92 ± 0.2 mg/dl glibenclamide 5 mg/kg), (248 ± 0.3 mg/dl Diabetic control) and (90 ± 0.3 mg/dl Normal control). Significant reduction in creatinine level of the nicotinamide-streptozotocin-induced diabetic rats treated with ASEMP was also recorded. Histopathology examination of the endocrine region of pancreas of the rats revealed restoration of pancreatic islet cells in the diabetic-ASEMP treated rats as the beta cell mass increased and necrotic changes was reduced significantly in contrast with the diabetic control group which showed degenerated pancreatic islet cells. Therefore, this study supports and recommends the exploration of the aqueous seed extract of *M. pruriens* for the management of type-2 diabetes.

Key words: *Mucuna pruriens*, glibenclamide, type-2 diabetes, anti-hyperglycemic activity, histopathology.

Introduction

The use of *Mucuna pruriens* in the treatment of various pathological conditions including diabetes mellitus is explored regularly. *Mucuna pruriens* is a leguminous plant belonging to the family of *Fabaceae* and consists of about 150 different species of climbing vines [1]. It is mainly grown in tropical Africa, India and the Caribbean as living mulch, green manure crop and ornamental plant [2]. The seeds of *M. pruriens* contain a number of bioactive substances such as tryptamine, alkaloids, alkylamines, steroids, flavonoids, coumarins, cardenolides, serotonin, nicotine, beta-sitosterol, squalene etc [3].

Diabetes mellitus is an endocrine disorder with multiple complications accounting for at least 10% of the total health care expenditure in many countries and more than 422 million people suffer from it worldwide. In India, the number of people with diabetes is projected to rise from 31.7 million to 79.4 million by the year 2030 [4]. Diabetes comes with various complications leading to hospitalization and results to significant financial burden [5].

Type-2 diabetes is the more common type which is non-insulin dependent hence, can be managed with dietary changes and proper medications. Though insulin therapy is used for management of diabetes mellitus, there are some drawbacks like insulin resistance [6], anorexia nervosa, brain atrophy and fatty liver after chronic treatment [7]. Conventional antidiabetic oral medications are effective but also have some unavoidable side effects on the patients. Thus, medicinal plants with antihyperglycemic activities can be employed as alternative source of antidiabetic agents due to their little or no adverse effects [8]. The limitations of currently available oral anti-diabetic agents either in terms of efficacy or safety, coupled with emergence of the disease into global epidemic have encouraged a concerted effort to discover drugs with natural active ingredients which can be used to manage diabetes more efficiently with no or less side effects [9].

In this study, we investigated the anti-hyperglycemic efficiency of two varieties of *M. pruriens var utilis* [IIHR selection 3 (S3), and *Arka Dhanvantari* (AD)] in nicotinamide-streptozotocin-induced diabetic *Wistar* rats. The aqueous seed extracts of *M. pruriens* were administered at different doses and significant reduction in the blood glucose and creatinine levels were observed. Also, the histopathology examination of the endocrine region of pancreas of the *Wistar* rats revealed restoration of pancreatic islet cells when compared with the diabetic control group which showed degenerated pancreatic islet cells.

Materials and methods

Source of seed samples and *Wistar* rats

Seeds of two varieties of *Mucuna pruriens:* (S3) and (AD) were collected from Indian Institute of Horticultural Research, (IIHR) Bengaluru. The plants were raised and maintained in the greenhouse for further analysis. The *Wistar* rats were obtained from small animal breeding centre, College of Veterinary and Animal Sciences, Kerala Veterinary and Animal Science University, Mannuthy, Thrissur, Kerala. Reg. No. 328/CPCSEA.

Housing and maintenance of animals

Male and female *Wistar* rats weighing about 200-250 g body weight were used in the study. The animals were acclimatized to the laboratory condition for 2 weeks. They were housed in polypropylene cages with dust free rice husk, humidity of 40-60% with 22±3 °C temperature, sound proof room at 12:12 hour light dark cycles with free access to standard feed and water *ad libitum* during the experiments.

The experiments conducted were in accordance with the rulings of the Committee for the purpose of control and supervision of experiments on animals at College of Pharmacy, Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore-641044, India, Registration number 1559/PO/a/11/CPCSEA and the study was permitted by the Institutional Animal Ethical Committee.

Acute toxicity study

The acute toxicity study was carried out by employing the fixed dose procedure following the guidelines No. 420 of OECD 2001. Nulliparous and non-pregnant female *Wistar* rats of 8-12 weeks old weighing 200-250 kg were used. The animals were fasted for 3-4 hours prior to testing with free access to water only. For sighting study two animals were administered with 300 and 500 mg/kg dose of *M. pruriens* aqueous seed extract respectively. As no mortality was observed, three animals were administered with 2000 mg/kg dose of *M. pruriens* aqueous seed extract for the main study.

Finally, fixed dose was selected for further *in vivo* studies. The animals were observed individually after dosing at least once during the first 30 minutes and periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days. Keen observations were done to determine the onset of signs, recovery and time to death. Observations included changes in skin and fur, eyes and mucous membranes, respiratory, autonomic and central nervous systems and behavioural pattern.

Nicotinamide-streptozotocin induction of type-2 diabetes to experimental animals and treatment with aqueous seed extract of *M. pruriens*

To induce the animals with type-2 diabetes, nicotinamide-streptozotocin administration was carried out. A single intraperitoneal (i.p.) injection of 110 mg/kg of nicotinamide was given first, then 15 minutes later i.p. administration of 65 mg/kg streptozotocin was given. Hyperglycemia was confirmed by elevated blood glucose levels at 72 hours and then on day 7 after injection. Only animals with fasting blood

glucose level greater than 200 mg/dl were selected for the anti-hyperglycemic study. Treatment of diabetic animals with ASEMP was carried out daily for 28 consecutive days.

Design of experiment

The experimental animals were divided into five groups, each consisting of six animals. Group I: Normal control rats (Drinking water); Group II: Diabetic control rats (Distilled water); group III: Diabetic rats administered with the aqueous seed extract of *M. pruriens* (ASEMP 200 mg/kg body weight); group IV: Diabetic rats (ASEMP 400 mg/kg body weight); group V: Diabetic rats (Glibenclamide 5 mg/kg). The fasting blood glucose level was recorded on day 0, 7, 14, 21 and 28 respectively. At the end of the experiments (day 28), blood samples were collected from the retro orbital plexus of the rats under light ether anesthesia, using glass capillaries and stored with or without disodium ethylene diamine tetraacetate for estimation of biochemical parameters. The blood samples were allowed to stand for about 15 minutes to clot, then centrifugation was done at 5000 rpm for 20 min for separation of serum. The serum was stored at -20°C until further analysis.

Biochemical analysis

Blood glucose determination

Blood glucose level of the experimental animals was estimated with whole blood using digital glucometer.

Lipid profile

The total cholesterol (TC) and creatinine levels of the *wistar* rats were estimated from serum using standard enzymatic diagnostic kits and compared with the control.

Histopathology examination

Dissection of animals for histopathology examination was carried out at the 28^{th} day of the study. The animals were killed by euthanasia (painless killing). The pancreatic tissues were dissected out and washed on ice cold saline immediately. A portion of pancreatic tissue was fixed in 10% neutral formal-saline fixative solution for histological studies. After fixation, the tissues were embedded in paraffin, solid sections were cut at 5 µm and various sections were stained with haematoxylin and eosin (H and E) as described by [10]. The slides were viewed at magnification of 250 X and photomicrographs were taken.

Analysis of data

For statistical analysis, data of the blood glucose were expressed as mean ± standard error. The data obtained were statistically analyzed using one-way analysis of variance (ANOVA) with Tukey's multiple comparison post hoc tests to compare the level of significance between control and experimental groups. The values of $p<0.05$ were considered as significant [11].

Results

Acute toxicity study

Nulliparous and non-pregnant female *Wistar* rats of 8-12 weeks old weighing 200-250 kg were used and no mortality was observed up to 2000 mg/kg body weight as recorded in table 1.

Table 1. Oral acute toxicity study of the aqueous seed extract of *M. pruriens*

Number of animals	Dose (mg/kg)	Observations
2	300	No mortality. No changes in skin and fur. No changes in eyes, mucous membranes, respiratory and central nervous systems were observed.
2	500	No mortality. No changes in skin and fur. Also. no changes in eyes, mucous membranes, respiratory and central nervous systems were observed.
3	2000	No changes in body weight. No changes in the general behavioural pattern of the animals were observed.

Biochemical assay

Blood glucose level was estimated with the whole blood using glucometer. Effect of ASEMP on fasting blood glucose in normal and diabetic treated *wistar* rats as recorded at 7 days intervals are shown in table 2. Significant reduction in blood glucose level of the ASEMP-treated rats was observed (from 286 ± 0.8 to 101 ± 0.3 mg/dl) in group 3 which received 200 mg/kg ASEMP while in group 4 which received 400mg/kg ASEMP, the reduction in blood glucose level was from 295 ± 0.6 mg/dl on the 7^{th} day of experiment to 94 ± 0.2 mg/dl at the 28^{th} day of experiment, as compared with normal control group (90 ± 0.3 mg/dl), diabetic control group (from 288 ± 0.4 to 248 ± 0.3 mg/dl) and glibenclamide treated group (from 285 ± 0.6 to 92 ± 0.2 mg/dl).

Table 2. Effect of ASEMP on fasting blood glucose in normal, diabetic and diabetic-treated *Wistar* rats recorded at 7 days interval

Experimental animals	**Category**	**Fasting blood glucose (mg/dl)**			
		Day 7	Day 14	Day 21	Day 28
Group 1	Normal control	90 ± 0.1	92 ± 0.4	91 ± 0.2	90 ± 0.2
Group 2	Diabetic control	288 ± 0.4	298±10.3	242 ± 7.2	248 ± 0.3
Group 3	diabetic rats (ASEMP 200 mg/kg)	286 ± 0.8	145 ± 0.8	109 ± 0.4	101 ± 0.3
Group 4	diabetic rats (ASEMP 400 mg/kg)	295 ± 0.6	108 ± 0.8	96 ± 0.2	94 ± 0.2
Group 5	diabetic rats (Glibenclamide 5 mg/kg)	285 ± 0.6	132 ± 0.4	94 ± 0.4	92 ± 0.2

Values are expressed as mean ± standard error (n=6). (n: number of animals in each group).

Effect of ASEMP on the total cholesterol and creatinine

The total cholesterol (TC) and creatinine levels of the rats were estimated from serum using standard enzymatic diagnostic kits as recorded in Table 3. High level of cholesterol in the NAD-STZ-induced diabetic rats was observed: group 2 (128.5 ± 1.8 mg/dl), group 3 (71.5 ± 0.1 mg/dl) group 4 (67.5 ± 0.2 mg/dl) and group 5 (69.2 ± 0.3 mg/dl) when compared with normal control: group 1 (59.2 ± 0.2 mg/dl). Significant reduction in creatinine level of the NAD-STZ-induced diabetic rats treated with ASEMP was

recorded. In group 3 and 4 which were treated with ASEMP 200 and 400 mg/kg body weight the creatinine decreased from 2.16 ± 1.2 mg/dl at day 7 to 1.55 ± 0.2 mg/dl at day 28 and from 2.19 ± 1.0 mg/dl at day 7to 1.30 ± 0.3 mg/dl at day 28 respectively as compared with the normal control group 1 (1.01 ± 0.1 mg/dl) and diabetic control group 2 (2.18 ± 1.1 mg/dl) at day 28 of the experiment.

Table 3. Effect of ASEMP on total cholesterol and creatinine in normal and diabetic-treated *Wista*r rats recorded after 28 days of treatment

Experimental animals	**Category**	**Total cholesterol (mg/dl)**	**Creatinine (mg/dl)**
Group 1	Normal control	59.2 ± 0.2	1.01 ± 0.1
Group 2	Diabetic control	128.5 ± 1.8	2.18 ± 1.1
Group 3	Diabetic rats (ASEMP 200 mg/kg)	71.5 ± 0.1	1.55 ± 0.2
Group 4	Diabetic rats (ASEMP 400 mg/kg)	67.5 ± 0.2	1.30 ± 0.3
Group 5	Diabetic rats (Glibenclamide 5 mg/kg)	69.2 ± 0.3	1.10 ± 0.1

Values are expressed as mean ± standard error (n=6). (n: number of animals in each group).

Histopathology examination

The effect of ASEMP on histopathology of normal, diabetic and diabetic-treated rats was assessed. Histopathology examination of the endocrine region of pancreas of the rats carried out after 28 days of the experiment revealed degenerated pancreatic islet cells in the non-treated diabetic *Wistar* rats when compared with the normal control which showed intact architecture of pancreatic islet cells. When the low (200 mg/kg) and high (400 mg/kg) doses of *M. pruriens* aqueous seed extract was administered, it

revealed the restoration of pancreatic islet cells in the diabetic-ASEMP treated groups as shown in figure 1.

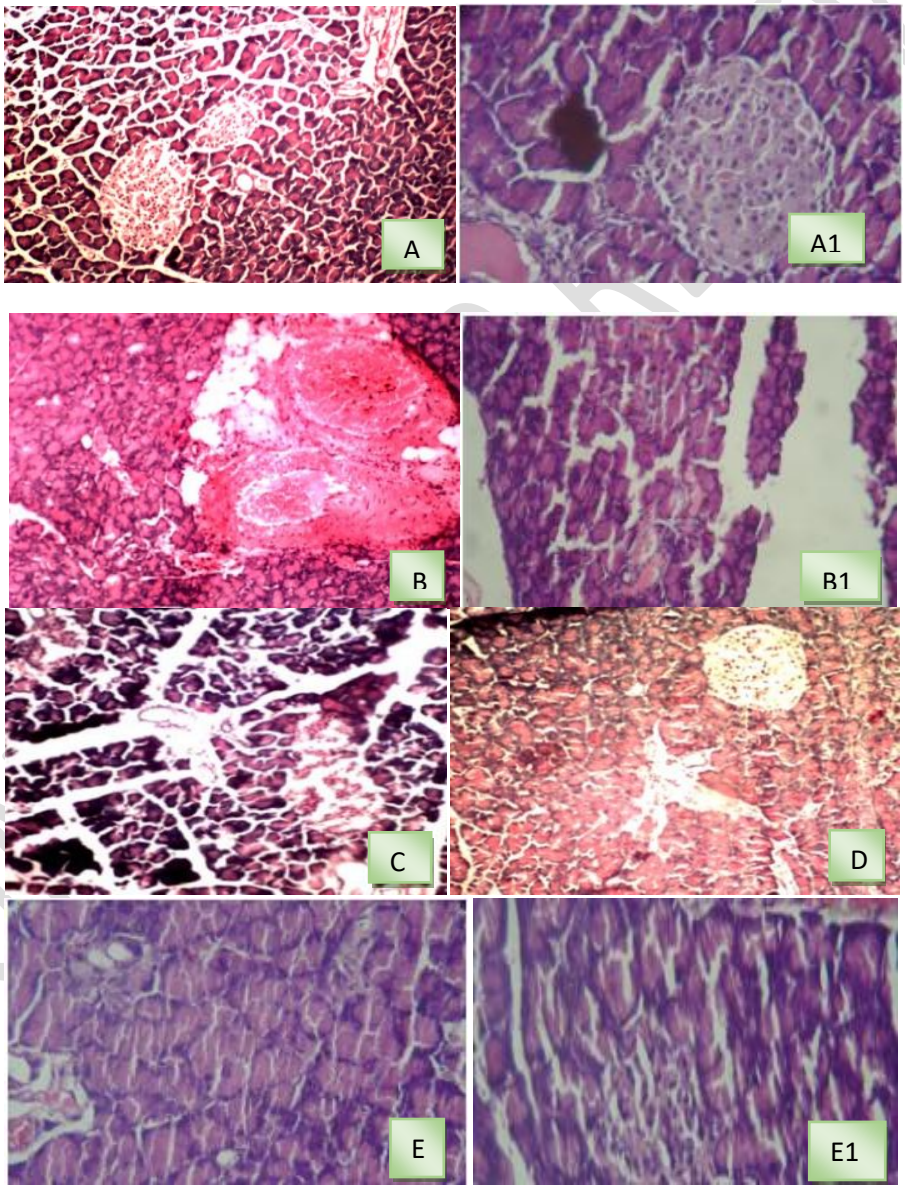

Fig 1. Histopathology examination of normal and diabetic rats under 250 X magnification.

A and A1: Micrograph showing pancreas of normal control rat.

B and B1: Micrographs showing pancreas of diabetic control rat with necrosis of pancreatic islet cells

C: Micrograph showing pancreas of diabetic *Wistar* rats administered with 200 mg/kg body weight of *M. pruriens* aqueous seed extract.

D: Micrograph showing pancreas of diabetic *Wistar* rats administered with 400 mg/kg body weight of *M. pruriens* aqueous seed extract.

E and E1: Micrographs showing pancreas of diabetic W*ister* rats administered with 5 mg/kg body weight of glibenclamide.

Discussion

Diabetes is characterized by high concentrations of blood glucose levels, which can cause serious complications, such as organ failures and/or destruction of the kidneys, eyes, and various cardiovascular diseases. Therefore, the treatment methods mainly focus on reducing fluctuations in blood glucose levels and their related complications. Ethnopharmacological surveys indicate that more than 1200 plants are used in traditional medicine for management of type-2 diabetes due to their hypoglycemic activities of which *Mucuna pruriens* is one of them [12].

The acute oral toxicity test carried out in the experimental animals showed no mortality up to 2000 mg/kg body weight when aqueous seed extract of *Mucuna pruriens* (ASEMP) was administered orally to the rats. Similar observation was recorded on acute oral toxicity study of *M. pruriens* seed extracts from 150 - 4000 mg/kg body weight of the experimental animals [13].

The oral administration of ASEMP showed anti-hyperglycemic effect against NAD-STZ- induced type-2 diabetes in the rats. Reduction in blood glucose level of the ASEMP-treated rats was observed (from 286 ± 0.8 mg/dl on the 7^{th} day of experiment to 101 ± 0.3 mg/dl on the 28^{th} day of the experiment) in group 3 which received 200 mg/kg ASEMP. In group 4 which received 400mg/kg ASEMP-treatment, the reduction in blood glucose level was from 295 ± 0.6 mg/dl on day 7 of the experiment to 94 ± 0.2 mg/dl on day 28 of the experiment as compared with normal control group (90 ± 0.3 mg/dl), diabetic control group (from 288 ± 0.4 to 248 ± 0.3 mg/dl) and glibenclamide-treated group (from 285 ± 0.6 to 92 ± 0.2 mg/dl). This result is similar to the report given by [14] on hypoglycemic effect of *M. pruriens* seed extract against STZ-induced diabetes in rats. It is well known that *Mucuna* is a rich source of dietary fiber which can reduce plasma glucose in humans with type-2 diabetes.

The effect of ethanol leaf extract of *M. pruriens* on blood glucose levels in alloxan-induced diabetic *Wistar* rats was also evaluated by [15] and they recorded a significant reduction in the fasting blood sugar levels in alloxan-induced diabetic groups treated with the leaf extract of *M. pruriens*. The hypoglycemic effect of the aqueous seed extract of *M. pruriens* was also investigated in normal, glucose load conditions and streptozotocin -induced diabetic rats and it was recorded that in normal and STZ diabetic rats, the aqueous seed extracts of *M. pruriens* (100 and 200 mg/kg body weight) significantly reduced the blood glucose levels after 2 hours of oral administration [16, 17].

Effect of ASEMP on the total cholesterol and creatinine in normal and diabetic-treated rats were determined after 28 days of treatment. High level of cholesterol in the NAD-STZ-induced diabetic rats was observed. The abnormally high concentration of cholesterol in the NAD-STZ-induced

diabetic rats may be due to insulin deficiency or insulin resistance. Insulin deficiency causes an increase in free fatty acid mobilization from adipose tissue which results in increased production of cholesterol. The level of total cholesterol in ASEMP-treated group was also observed to decrease significantly, this may be due to the presence of squalene in the extract [18].

The creatinine level was measured, since diabetes also causes renal damage due to abnormal glucose regulation. It was observed that the creatinine level reduced from 2.16 ± 1.2 mg/dl on day 7 to 1.55 ± 0.2 mg/dl on day 28 in group 3 and from 2.19 ± 1.0 mg/dl on day 7 to 1.30 ± 0.3mg/dl mg/dl on day 28 in group 4, when compared with the normal control: group 1 (1.01 ± 0.1 mg/dl) and diabetic control: group 2 (2.18 ± 1.1 mg/dl) at day 28 of the experiment. Therefore, the significant reduction in the creatinine level of these NAD-STZ-induced diabetic rats indicated the reno protective role of ASEMP. The extracts were highly effective in managing the complications associated with type-2 diabetes mellitus. These findings are in agreement with the results of a study on streptozotocin-nicotinamide induction of diabetes in rats [19]. Similar report was given by [20, 21] on natural oral anti-diabetic agents.

Histopathology examination of the endocrine region of pancreas of the rats revealed degenerated pancreatic islet cells in the non-treated diabetic *Wistar* rats when compared with the normal control which showed intact architecture of pancreatic islet cells. However, when the low (200 mg/kg) and high (400 mg/kg) doses of *M. pruriens* aqueous seed extract was administered, it revealed the restoration of pancreatic islet cells in the diabetic-ASEMP-treated groups. This shows the remedial and non-disruptive property of ASEMP on the pancreas of type-2 diabetic rats. Similar observation was made by [22] on anti-diabetic activity of benzopyrone analogues in nicotinamide-streptozotocin induced type-2 diabetes in experimental rats.

Conclusion

Recent advances in research towards traditional medicine have significantly increased the development of novel drugs for the management of diabetes and its complications. The reduction in blood glucose and creatinine levels recorded from the treatment of the nicotinamide-streptozotocin-induced diabetic *Wistar* rats using the aqueous seed extract of *M. pruriens* and the restoration of pancreatic islet cells revealed that *M. pruriens* possess anti-hyperglycemic efficiency. The acute toxicity study also revealed that the aqueous seed extract is relatively safe. Therefore, this study supports the exploration of *M. pruriens* seed extract for management of type-2 diabetes.

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