



Original Research Paper

EVALUATION OF ANTI-HYPERGLYCAEMIC EFFECT OF *MUCUNA PRURIENS* IN DIABETIC RATS

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ABSTRACT

The antidiabetic potential of the alcohol root extract of *Mucuna Pruriens* is a medicinal plant widely used in more than 34 Ayurvedic preparations, was evaluated in the streptozotocin induced type 2 diabetic model. Graded doses of the root extract were administered to normal and experimental diabetic rats for 15 days. A significant ($p < 0.05$) reduction was observed in the fasting blood glucose levels of normal as well as diabetic rats. Body weight, serum lipid profiles were estimated in extract-treated normal and diabetic rats. Significant results were observed in the estimated parameters, thereby justifying the use of the plant in the treatment of diabetes mellitus.

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1. INTRODUCTION

Diabetes is a well-known chronic disorder characterized by many metabolic changes in the body as a result of insufficiency in secretion of insulin and peripheral resistance to insulin. The worldwide prevalence of diabetes is on the rise due to obesity, poor feeding habits and sedentary lifestyle. There were about 518,400 known cases of diabetes in India. Diabetes presents with long term complications that lead to damage of the retina, kidney, and liver. It can also lead to cardiovascular diseases. Ongoing planned investigations have given unequivocal proof on the pivotal job of delayed hyperglycaemia in the advancement of constant diabetic inconveniences.^[1]

In view of the many metabolic changes that occur in diabetes, many drugs are employed to tackle the cause, complications and symptoms at the same time. Treatment of diabetes is challenging as the pathological mechanisms of diabetes and its complications are multidirectional and complicated.^[2]

Plants have been an alternate source of medicines for many over the years. Plants may have a significant role to play in diabetes. Apart from their ease of accessibility, plants are known to possess anti-oxidative, anti-inflammatory, analgesic and anti-hyperglycaemic activity. All of these

properties will tackle key components of diabetes and possibly its complications.^[3]

Mucuna Pruriens is very common and easily accessible in India. It has been proven to have low toxicity in chronic and acute administration of high doses in rats. The plant also possesses anti-inflammatory and *in vitro* antioxidant properties. This study however seeks to further study the effects of an aqueous extract of the stem bark extract in streptozotocin-induced diabetic rats. This will provide further pharmacological information regarding the anti-diabetic potential of the plant towards drug discovery. The hypoglycaemic properties of the aqueous extract from the plant provides the basis for further research into the possible anti-hyperglycaemic. It is also a cheaper alternative to current treatments. This research aims at investigating the anti-hyperglycaemic effect of the aqueous extract of *Mucuna Pruriens* in diabetic rat models.

2. PLANT PROFILE

Mucuna pruriens also known as velvet beans is a tropical climbing legume belonging to the family fabaceae. It is developed in Asia, America, Africa, and the Pacific Islands, where its units are utilized as a vegetable and medicinal purpose for human utilization.

Chemical constituents: It consists of polyphenols (tannins), phytate, cyanogenic glycosides, oligosaccharides, saponins, lectins, and alkaloids. The major component of *Mucuna pruriens* is L-dopa (5%), along with minor amounts of methylated and non-methylated tetrahydroisoquinolines (0.25%). Mucunine, mucunadine, prurienine, and prurieninine are four are four alkaloids.

Traditional Uses: It is used as an antioxidant agent, anti-inflammatory agent, antiviral agent, antimicrobial agent. Studies have shown that it effectively treats Parkinsonism, epilepsy and also it is hostile to cancer causing agents.

3. MATERIAL AND METHODS

The HPLC analytical graded stem bark crude powder of *Mucuna Pruriens* was collected from Bio extract Pvt. Ltd, Bangalore. The dried stem bark was pounded with a sledge factory into powder which was then removed by decoction (bubbled in refined water for fifteen minutes). The decoction was then separated, permitted to cool and solidified. It was then kept at roughly - 20°C, the stop dried concentrate (a dark colored powder) extract was then gathered into a glass holder and put away in a dedicator. The last yield was 15.2% w/w.^[4] Phytochemical screening for the presence of alkaloids, tannins, saponins, triterpenoids, flavonoids, volatile oils, phenolic and glycoside was done according to various tests specified in literature.^[5]

4. EXPERIMENTAL SETUP

Animals

Albino wistar rats (90-120 g) were obtained from the CPCSEA approved animal house, Sai nath agency, Hyderabad. The animals were allowed to acclimatize and adapt to the new environment a week before the study commenced. They were put in cages of five rats each in stainless steel cages (34 cm × 47 cm × 18 cm) with wood shavings as bedding. The room for the cages was adequately ventilated at normal room temperature (22–26 °C). The relative humidity of 60–70 %with a natural light-dark cycle was maintained during the period. Good sanitation practices were observed with regular cleaning of cages and change of bedding. The rats were fed with rat feed and given clean water *ad libitum*. The processes and procedures employed during the experiment as per OECD and CPCSEA guidelines. The procedures for the research work was approved by the animal ethics committee.^[6]

Group- I (Normal Control)- Administered vehicle serves as Normal control.

Group- II (Negative Control)- Administered STZ (55mg/kg i.p.) Serves as diabetic control

Group- III: Diabetic rats treated with *Mucuna Pruriens* stem bark aqueous extract (250mg/kg,p.o.)

Group- IV: Diabetic rats treated with *Mucuna Pruriens* stem bark aqueous extract (500mg/kg,p.o.)

Group- V: Administered Reference standard, Insulin (1U/kg, i.p.)^[7]

Induction of Diabetes Mellitus

Induction of diabetes was done 1 week after arrival of rats (after acclimatization). Animals to be induced were fasted for 16 hours and

subsequently weighed and given a single intra-peritoneal injection of STZ (55 mg/kg) already dissolved in a freshly prepared citrate buffer of pH 4.5. Rats in control group were given only citrate buffer of equal volume. The rats were allowed access to feed and water *ad libitum* after the injection.

Three (3) days after the injection, FBS was measured for all the animals using a blood glucose monitor. FBS was measured at 7 am in the morning, one hour before feeding. Animals with values greater than 11 mmol/L were considered to be diabetic.^[7]

Oral glucose tolerance test

All STZ injected rats were fasted overnight and orally administered with bolus glucose load of 2 g/kg at 08:00 AM. Blood samples were periodically measured from the tail vein at 0, 30, 60, 90, and 120 min after administration of the glucose. The levels of blood glucose were evaluated by using a glucose monitor. All rats with a blood glucose level above 11 mmol/L after 2 hours were considered to be diabetic. This test is a confirmatory test for diabetes.^[8]

Preparation of drugs

Insulin human (rDNA) was administered as reference drug was serially diluted with Normal saline to obtain the minute quantities needed. This solution was freshly prepared daily before administration subcutaneously.

Drug administration

The control and diabetic control group received no treatment except food and water. Insulin group received a subcutaneous injection of Insulin human (rDNA) twice daily. Animals received a daily gavage at 7:30 am, 30 minutes before feeding. Drug administration was done every day for 8 weeks before animals were sacrificed.

Fasting blood sugar (FBS) determination

FBS of animals were measured twice weekly and the weekly average calculated throughout the eight (8) weeks. Fasting blood sugar was taken at 7am every morning one hour before feeding using the OneTouch Select plus glucometer.^[9]

Determination of percentage change in body weight and relative organ weight

The first and final weights of the rats were recorded on the day of induction of diabetes and on the last day of the experiment when the rats were sacrificed. The percentage changes of body weight of each group were calculated using the formula below:

The harvested liver, spleen, kidney, brain and heart were washed with normal saline, dried with tissue paper, and weighed. The weight of the excised organs with respect to the body weight of the rat at the end of the experiment was calculated.^[9]

HISTOPATHOLOGY

Portions of the organs were selected into labelled tissue processing cassettes and processed into paraffin blocks. Each was passed through ascending grades of alcohol (70%, 80%, 90% and absolute) and further two changes of absolute alcohol for dehydration, cleared in three changes of xylene and finally infiltrated and embedded in paraffin wax. Five-micron sections were cut from each block, mounted on microscope slides and stained by the haematoxylin and eosin method.^[9]

Statistical Analysis

Each result is presented as mean ± SEM (standard error of mean). Statistical analysis of the data were performed with one-way analysis of

variance (ANOVA) followed by the Tukey post hoc comparison test. Dose dependent effects were evaluated using correlation coefficient. Significant differences were set at p- values lower than 0.05.^[4]

5.RESULTS AND DISCUSSION

TABLE 1 Phytochemical screening of the aqueous extract of *Mucuna Pruriens*

CONSTITUENT	RESULT
Alkaloids	+
Saponins	+
Taninns	+
Phytosterols	+
Phenolics	+
Volatile oils	-
Terpinoids	+
Flavonoids	+
Glycosides	+
Absent:-	Present:+

Induction of Diabetes

The Fasting blood sugar of both control and treatment group were measured before and 72 hours after the injection of STZ (55 mg/kg bodyweight). There was a significant increase in fasting blood sugar levels for the STZ-treated group ($P < 0.0001$) in comparison to the control group. Subsequently the vehicle group (untreated) maintained a high fasting blood sugar over the 8 week period significantly higher than the control group.^[7]

Anti hyperglycaemic Activity

The administration of 500 mg/kg gave a significant reduction in fasting blood sugar levels on the 4th week. The total fasting blood sugar activity (calculated as AUCs) showed that the doses gave no significant reduction in insulin over 8 weeks. The administration of insulin (0.1-1.0 IU/kg) showed a significant reduction in the FBS for only 1IU/kg dose of insulin on the 3rd and the 8th week. All other doses of insulin did not give any significant reduction in fasting blood sugar compared to the control ($P > 0.05$). The total fasting blood sugar activity showed that the highest of insulin (1 IU/kg) gave a significant reduction in insulin over 8 weeks.^[7]

Percentage change in body weight

The percentage change in body weight was calculated by finding the difference between final and the initial weight and expressing it as a percentage of the initial weight. Induction of diabetes caused a significant weight loss in the vehicle and doses compared to the control after 8 weeks of treatment.^[7]

TABLE 2 Effect of diabetes on relative organ weights of diabetic rats for 8 weeks

Organs	Relative organ weight (%)				P-VALUE
	Vehicle	Negative control mg	SNE250 mg	SNE 500 mg	
Liver	6.00 ±0.58	3.17±0.20	4.45±0.38	4.28 ±0.31	0.02

Values are means ± SEM (n=5). For P-value, * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ compared with vehicle-treated group (B) (one-way analysis of variance followed by Bonferroni's post hoc test)

TABLE 3 The effect on blood biochemistry in STZ –induced diabetic rats after 8-week administration

Parameter	Normal control	Negative Control	Standard	Test- I	Test-II
Urea (mmol/L)	14.62±2.1	9.07±0.5	13.59±0.2	11.65±.36	12.68±2.2
Creat(mmol/L)	34.80±2.2	75.08±4.8	32.43±4.5	28.43±4.1	30.60±5.2
Tot. Chol.	2.17±0.2	2.10±0.2	2.01±0.2	1.71±0.6	2.00±0.3
Triglycerides	2.55±0.6	2.45±0.4	2.100±0.2	1.42±0.4	1.90±0.8
HDL(mmol/L)	1.06±0.3	0.81±0.1	1.00±0.1	0.80±0.1	0.95±0.1
LDL(mmol/L)	0.48±0.4	0.68±0.1	0.47±0.4	0.35±0.5	0.40±0.6

Data are mean ± SEM (n = 5). * $P \leq 0.05$, ** $P \leq 0.01$, and *** $P \leq 0.001$ compared to control group (one-way ANOVA followed by aBonferroni's post hoc test)

The study described demonstrates that the aqueous extract of *Mucuna Pruriens* possesses anti-hyperglycaemic activity in STZ-induced diabetic rats. This may corroborate previously discovered properties, which are analgesic and anti-inflammatory properties. Albino wistar rats were injected with STZ (55 mg/kg) to induce diabetes. STZ acts as a nitroso-urea alkylating agent that attacks the DNA of beta pancreatic cells. This causes beta cell death leading to a decreased insulin level hence diabetes. After 8 weeks of extract administration the animals were assessed to find out if the extract had any significant effects on biochemical markers for diabetes. A number of effects were observed.

Phytochemical screening identified the presence of alkaloids, saponins, tannins, phenolics, terpenoids, flavonoids and glycosides in the aqueous extract. Thus, the hydro aqueous solvent (i.e 30% water, 70% ethanol) had the ability to absorb a lot of bioactive compounds from the whole plant mixture. These phytochemicals could be contributing individually or synergistically to give the observed effects. There are likely to be bioactive compounds which may have anti-oxidant, anti-hyperglycaemic and anti-inflammatory effects in the extract.^[7]

Weight gain or loss is an important parameter in the diagnosis and assessment of the progression of the diabetes. Weight loss in early stages of diabetes, especially type 1 is attributed to the lack of insulin. However, the highest dose of the extract (500 mg/kg) produced a mild gain in weight. 500 mg/kg was the only dose of the extract that showed a positive percentage change in bodyweight though significantly lower than the control. It's possible that the extract provided a form of nutritive/supplementary value. The plant has already been known to be used as food by both humans and in livestock. The slight weight gain could also be as a result of its anti-hyperglycaemic effects.^[8]

A relatively large liver was observed in the vehicle group, significantly higher in comparison with the SNE treated groups. The increase in weight of the liver is in direct correlation to an increase in liver size. It can also be inferred that the extract prevented hepatomegaly by preventing liver inflammation or reducing hyperglycaemia or both.

Free radicals in streptozotocin-induced tissue damages in diabetic rats has been demonstrated already. Hepatocytic proteins, lipids and DNA are

among the cellular structures that are primarily affected by reactive oxidative species. Liver enzymes are also usually released into the blood when such damages occur, and have been measured to assess the ability of certain extracts to reduce liver damage in diabetes. In this study there was a significant increase in the levels of these enzymes for the vehicle group.^[9]

The decrease of creatinine in all the diabetic rats is that the diabetes affected the muscle mass due to gluconeogenesis. The lower the muscle mass of the rats the lower the amount of creatine/creatinine produced. Gluconeogenesis leading to weight loss has already been mentioned above.

Significantly low albumin levels were observed for the diabetic untreated vehicle group. Since the extract group had albumin levels close to normal it suggests that the extract was able to reduce inflammation of the liver. This may be further buttressed by the fact that haematology results also showed a significantly high level of eosinophils in the vehicle group.

Histopathology also showed a change in the tissue structure of the liver for the vehicle group. The central veins were slightly dilated and distorted. There were also large interstitial spaces seen in liver tissue. Increase in blood supply to the liver usually leads to an increase in hydrostatic pressure of the blood flowing to the liver. Increase in blood flow to the liver can be associated with inflammation. The fluid build-up was responsible for the large spaces seen in between the liver tissues and is characteristic of increased hydrostatic pressure seen in liver inflammation and cirrhosis.^[9]

There were mild leucocytic infiltrations in the kidneys for both extracts treated and untreated diabetic (vehicle) groups. Leucocyte infiltrations in

the kidney are usually attributed to inflammation. There were also a few blood congestions seen. There were however no serious pathological conditions observed such as tubular or glomerular necrosis. Possible diabetic nephropathy could still be in the nascent stage. Similarly, lung histopathology showed thickened alveolar and leucocyte infiltrations for all groups of diabetic rats. Allergic and inflammatory reactions usually result in lung leucocyte infiltrations. The presence leucocytes in lungs however, may not be unusual as it is part of the natural defence system against infection. Brain histopathology showed mild signs of astrogliosis in all groups. Demyelination or any other abnormalities were however not prominent. Astrogliosis is the mechanism employed by the CNS for Défense and repair against injury. It is characterized by an increase in astrocytes in the area of damage. Diabetes is known to play a role in CNS neuropathy and gliosis. The mild astrogliosis seen in all groups may be a sign of developing CNS neuropathy. There was however no significant difference seen amongst all diabetic groups. There was also no significant pathological damage observed.^[9]

6. CONCLUSION

The aqueous extract of plant extract showed anti-hyperglycaemic and a possible anti-inflammatory activity in streptozotocin-induced diabetes in rats. It also exerted various effects on blood biochemistry and histopathology which corroborated its anti-hyperglycaemic.

7.CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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