ISSN:0975-5772

Murugan M. *et al* / Journal of Pharmaceutical Science and Technology Vol. 1 (2), 2009, 69-73

Hypoglycemic and hypolipidemic activity of leaves of *Mucuna pruriens* in alloxan induced diabetic rats

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ABSTRACT:
Ethanolic leaf extract of *Mucuna pruriens* was isolated with different fractions and were subjected to preliminary qualitative chemical investigations. The chloroform fraction showed the presence of glycosides and alkaloids. Acute toxicity was carried out in wister rats and the fractions were found to be safe upto higher doses of 2000mg/kg body weight. The chloroform fraction was screened for its hypoglycemic and hypolipidemic activity, the result was comparable with the positive control glibenclamide.
Key words: Glibenclamide, Hypoglycemic, Hypolipidemic, *Mucuna pruriens.*

INTRODUCTION:

Diabetes mellitus is the most common endocrine disorder. More than 150 million people suffering from it worldwide and this is likely to increase to 300 million by the year 2025 [1]. More than one-fifth of them are Indians, according to the International Diabetes Federation, India has been declared as "Diabetic Capital of the World" at the recent Conference in Paris [2]. Plants have been used as sources of drugs for the treatment of diabetes in developing countries where the cost of conventional medicines is a burden to the population [3].

Diabetes is a metabolic disorder associated with many other metabolic functional alterations. The main objective of the study was to assess the anti diabetic potential of leaves of *Mucuna pruriens.* The plant is commonly called as "Ponnaikkali" in tamil. Hence the present study has been aimed to investigate the fractions of ethanolic extract of *Mucuna pruriens* in terms of controlling the blood glucose levels and effectiveness on various biochemical parameters. The study also includes the preliminary photochemical screening, acute toxicity studies and evaluation of hypoglycemic and hypolipidemic activity.

MATERIALS AND METHODS:-

The leaves of *Mucuna pruriens were* collected from the local areas of Sivagangai district in Tamilnadu, India.

EXTRACTION:-

The leaves were dried under shade, crushed into coarse powder. The powder was loaded in to the Soxhlet extractor in 5 batches of 200gms each and was subjected to extraction for about 48 hours with ethyl alcohol (95%). After extraction the solvent was distilled off and extract was concentrated on water bath to a dry residue.

FRACTIONATION:-

The concentrated 95% ethanolic extract was dispersed in 250ml of distilled water and subjected to successive fractionation with toluene, chloroform, ethyl acetate and n-butanol. Each fraction was washed with water, then dried over anhydrous sodium sulphite (Na2SO3) and concentrated to small volume and then evaporated to dryness. All the fractions were kept in a desiccator and stored in refrigerator for chemical investigation and pharmacological studies.

The ethanolic extract and individual fractions were subjected to qualitative chemical investigations for the identification of the phytoconstituents viz. glycosides and alkaloids.

ANIMALS:-

Wister albino rats weighing above 150 gms were housed in large spacious hygienic polypropylene cages and animals had 12+1 hour day and night cycle. The animals were allowed to standard pellet diet and tap water *ad libitum.* The husk for the purpose of keeping as a bed to the animals was cleaned and autoclaved. Before the animals were kept in the polypropylene cages were sterilized along with water feeding bottles.

ACUTE TOXICITY STUDIES:-

Albino rats of either sex weighing above 150 gms were used to determine LD_{50} of various fractions. The gum acacia 2% was used as a vehicle to suspend various fractions were found to be safe upto the higher dose level of 2000mg/kg of body weight.

PREPARATION OF DIABETIC RATS:-

Experimental diabetes in rats was induced by injecting alloxan monohydrate intraperitoneally at a dose of 120mg/kg body weight in ice cold citrate buffer pH 4.5[9].

After 72 hours of blood was collected from the tail vein under mild ether anesthesia of all surviving rats and blood glucose levels were determined colorimetrically by using auto analyzer microlab 200 with blood sugar levels of 200-350 mg/dl were considered as diabetic and were employed in the study.

The project was undertaken with prior approval from the animal ethics committee. Utmost care was taken to ensure that the animals were treated in the most humane and ethically acceptable manner.

HYPOGLYCEMIC SCREENING:-

The rats were randomly divided into 6 groups of six animals each. Group I served as diabetic control and received 0.3% normal saline orally. Group II served as positive control and received Glibenclamide (10 mg/kg). Group III to VI received suspensions of fractions, orally at a dose of 150 mg/kg. The treatment was continued for 8 days by administering the respective drug fractions or 0.3% normal saline, twice daily.

Blood samples were collected by tail vein method and analyzed for acute and sub acute treatment. The blood glucose levels were estimated at 0, 1, 3 and 5 hrs after the extract administration. The administration of normal saline, Glibenclamide (10 mg/kg body weight) and the test extracts were continued for 7 days, once daily. Blood samples were analyzed for blood glucose levels after 1, 3, 5 and 7 days of administration.

HYPOLIPIDEMIC ACTIVITY:

Blood samples were collected and centrifuged to separate serum for estimation of lipid profile. Total cholesterol, total protein was analyzed from serum using standard procedures in an autoanalyzer Microlab 200 using Ecoline kits. Triglycerides were determined using Hantzsch condensation method.

STATISTICAL ANALYSIS:-

Data were expressed as Mean ± SE and analyzed statistically using one way ANOVA by Dunnet's multiple comparision test. The result of biochemical estimations were analyzed statistically and compared with those of control using students t- test[10]. The level of significance was fixed at $P<0.05$.

RESULTS:-

EFFECT ON BLOOD GLUCOSE:-

Alloxan (120 mg/kg, i.p.) elevated the blood glucose levels which was partially restored or improved upon the administration of chloroform fraction of leaf extract of *Mucuna pruriens.*

Table I: Effect of chloroform fraction of *Mucuna pruriens on* blood glucose level in alloxan induced diabetic rats (acute treatment).

Group	Treatment	Dose	Blood glucose mg/dl (mean ± SEM)				
			0 hr	1 hr	3 hr	5 hr	7 hr
I	Vehicle control	2ml saline	91.5 ± 1.0	91.7 ± 0.6	91.3 ± 1.0	91.1 ± 1.0	91.1 ± 1.0
II	Alloxan control	120 mg/ kg	338.8 ± 4.8**	343.9 ± 4.0**	355.0 ± 6.9**	360.8 ± 6.2**	367.3 ± 5.8**
III	Extract	250 mg/kg	337.8 ± 6.7**	311.4 ± 5.7**	255.0 ± 5.6**	219.8 ± 7.5**	183.8 ± 9.1**
IV	Glibenclamide	10 mg/kg	320.0 ± 3.1**	303.92 ± 5.7**	227 ± 11.0**	187.1 ± 3.5**	176.8 ± 4.1**

"+" denotes increase and "-"denotes decrease in hypoglycemic activity.
P < 0.01 ** Values are given average blood glucose (mg) ± SEM (Standard Mean Error), n = 6.

Table II: Effect of chloroform fraction of *Mucuna pruriens on* blood glucose level in alloxan induced diabetic rats (sub-acute treatment).

Group	Treatment	Dose	0 day	1st day	3rd day	5th day	7th day
I	Vehicle control	2 ml saline	91.5 ± 1.0	91 ± 0.9	90.5 ± 0.8	90.6 ± 0.8	91.0 ± 0.8
II	Alloxan control	120 mg/ kg	338.8 ± 4.8*	378.5 ± 5.6**	388.5 ± 5.7**	397.8 ± 6.0**	406.1 ± 6.0**
III	Extract	250 mg/kg	337.8 ± 6.7**	179.6 ± 3.6*	150.0 ± 1.7**	119.5 ± 2.2**	103.8 ± 0.7**
IV	Glibenclamide	10 mg/kg	320.0 ± 3.1**	158.3 ± 1.8**	132.3 ± 2.1**	118.5 ± 2.1**	98.0 ± 1.3**

"+" denotes increase and "-"denotes decrease in hypoglycemic activity.
P < 0.01. ** Values are given average blood glucose (mg) ± SEM (Standard Mean Error), n = 6.

TableIII: Effect of chloroform fraction of *Mucuna pruriens on* lipid profile in alloxan induced diabetic rats.

Treatment	Cholesterol (mg/ dl)	Triglycerides (mg/dl)	Total Protein (g/dl)
Normal control	66 ± 4.6	97.6 ± 2.3	6 ± 0.5
Alloxan control (120 mg/kg)	177.6 ± 4.6	184.6 ± 12.4	3 ± 0.5
Chloroform Extract(250mg/kg)	69.6 ± 3.4	108.3 ± 10.9	7.3 ± 0.3
Glibenclamide (10 mg/kg)	71 ± 7.8	111.3 ± 9	5 ± 0.5

Glucose levels were found to be significantly increased till 9th day after alloxan administration and thereafter decreased on 12th day. Decrease in Serum glucose may be due to the regeneration of β cell of the pancreas which was destroyed by alloxan. Administration of the extract produced a significant ($p<0.001$) decrease in the serum glucose as compared to diabetic control group.

HYPOLIPIDEMIC ACTIVITY:

A significant decrease ($p<0.001$) in the cholesterol, triglycerides levels were observed. Chloroform fraction controlled the elevation of lipid profiles, cholesterol and triglycerides significantly in comparison with the standard control Glibenclamide.

DISCUSSION:-

The average percentage yield of ethanolic (95%) extract of leaves of *Mucuna pruriens* was found to be 15.80% of w/w and the corresponding value for toluene, chloroform, ethyl acetate and n- butanol fractions. Glycosides and alkaloids were found to be present in ethanolic extract of chloroform fractions.

Alloxan has been shown to produce hypoglycaemia which seemed to retain partial beta cell activity and ineffectiveness in severe diabetes may be due to complete destruction of beta cells. Insulin deficiency leads to various metabolic alterations in the animals viz increased blood glucose, increased cholesterol, increased levels of alkaline phosphate and transaminases [5-7].

The ethanolic extract was also found to normalize of blood glucose levels which resulted in significant reduction in levels of plasma cholesterol, free fatty acids and plasma apo protein. Treatment with Glibenclamide, showed an increase in the body weight of diabetic rats probably due to improvement in glycemic control. Glibenclamide may suppress a hepatic gluconcogenesis, stimulate glycolysis or inhibit glucose absolution from the intestine. Oliver[8] listed glycosides, alkaloids, flavonoids and steroidal compounds as active ingredients in hypoglycemic plants.

Hyperlipidemia is a recognized complication of diabetes mellitus characterized by elevated levels of cholesterol, triglycerides and changes in lipoprotein composition. A marked increase in serum cholesterol and triglycerides levels was observed in diabetic rats. Treatment with chloroform fraction of *Mucuna pruriens* reduced the cholesterol and triglycerides level.

CONCLUSION:

From this study, we can state that the chloroform fraction of *Mucuna pruriens* has beneficial effects on blood glucose levels as well as improving hyperlipidemia and other metabolic aberrations. Further pharmacological and biochemical investigations are underway to elucidate the mechanism of action. The above observations show that the ethanolic extract of leaves of *Mucuna pruriens* possesses antidiabetic activity and it may be used in diabetic conditions with or without cardio-vascular complications.

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