Research Article

Qualitative Phytochemistry and Acute Oral Toxicity Testing of the Methanol Extract of Mucuna Pruriens Seeds in Albino Mice

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ABSTRACT

This study was carried out to identify the various phytoconstituents present in the seeds of Mucuna pruriens and also to find its acute oral toxicity. Fresh seeds were procured, shade dried and methanol extract was prepared by Soxhlate method. The yield obtained was 8.67%wt/vol. The phytochemical studies were done by standard protocols of pharmacognosy and the acute toxicity effects by OECD guideline 423. 2000mg/kg of methanol extract was administered by oral route in albino mice weighing about 18-25gm. The wellness parameters, biochemical parameters, haematological parameters were assessed and histopathological studies were done to know the toxic effects of the methanol extract of seeds of MP if any. The phytochemical studies confirmed the presence of Carbohydrates, Proteins & amino acids, glycosides, alkaloids, phytosterols, flavonoids, saponins, tannins and phenolic compounds. The toxicity study results showed that all the parameters were normal and there was no associated morbidity or mortality. Thus we conclude that the methanol extract of Mucuna pruriens has a LD50 value above 2000mg/kg and the drug is safe under therapeutic doses.

Keywords: *Mucuna pruriens Linn*, Phytochemistry, Acute toxicity, Histopathology.

INTRODUCTION

Many herbal products which is primarily based on traditional medicine are important sources of income, food and health care. In many of the developing countries and even the developed countries, traditional system of medicine plays a vital role because of its availability and affordability. Due to the key factors like low cost and easy availability, traditional system of medicine like Ayurveda and siddha are widely spread even in remote areas as compared to modern medicine.[1] In places like in Africa and China, traditional medicine accounts for the health care needs of nearly 80% and 40% respectively. Even in developed countries like USA at least 42% of the population has used traditional medicine at least once in their life time.[2]

Mucuna pruriens Linn. (MP) (Fabaceae) is a herb used in traditional system of Indian medicine.[3] It is legume of climbing variety found in Asia, Africa and various parts of America. In India it is predominately found in Southern regions of Tamil Nadu and Kerala.

The common name is "cow itch", "velvet bean" and in Tamil it is called "Ponaikalli".[4] All the parts of the plants are of medicinal value.

The roots of the plant are used for nephropathy, strangury, dysmenorrhoea, amenorrhoea, elephantiasis, dropsy, neuropathy, ulcers, and fever and as febrifuge and tonic. Leaves of this plant are regarded as aphrodisiac and tonic and useful in ulcers, inflammation, helminthiasis, cephalalgia and general debility. Seeds of this plant are used in snakebite, sexual debility, cough, tuberculosis, impotence, rheumatic disorders, muscular pain, gonorrhoea, sterility, gout, delirium, dysmenorrhea, diabetes, and cancer.[5,6]

Several authors have proposed that this plant possesses antiparkinson effect (contains L-DOPA), antidiabetic, antioxidant, antiepileptic, anti-inflammatory and also neuroprotective action.[7] The leaves and pods are used by certain tribal groups in Nigeria as food.[8] In India it is used as a nervine tonic and as an aphrodisiac.[9] Various food companies also uses the seeds of the Mucuna Pruriens as soup thickeners.[10] Studies of the past have suggested that the Mucuna pruriens seeds may cause hepatocellular destruction in a dose dependent manner.[11] This study has evaluated the phytochemical composition and acute toxicity with histopathological studies of the methanol extract of the seeds of Mucuna pruriens.

MATERIALS AND METHODS

Preparation of the plant extracts material

The seeds of *Mucuna pruriens* were procured from the local market and were authenticated. The seeds were then washed thoroughly in water and later in distilled water to remove impurities.

Later they were shade dried for 7-10 days and then pulverized into fine powder. Extraction was performed by Soxhlet method using around 400 ml of methanol for 80 gm of the seed powder. The extract was later concentrated using rotary flash evaporator. The percentage yield was 8.67% wt/vol. The extract was

packed in air-tight container and refrigerated at 8°C for further use.

Preliminary phytochemical studies

The preliminary phytochemical studies were conducted to ascertain the basic phytochemicals present in the extract using a standard protocol.[11]

Test for reducing sugars

Molish test

The extracts were treated with 2-3 drops of 1% alcoholic napthol and 2ml of concentrated sulphuric acid was added along the sides of the test tube. Presence of carbohydrates was indicated by the presence of purple ring between two layers.

Fehlings test

5ml of extract was boiled with 5 ml of Fehling's solution in a test tube and heated for twenty minutes. Development of brick red colour indicated the presence of reducing sugars.

Benedicts test

5ml of the extract solution and 5 ml of Benedicts reagent were mixed in a test tube and heated for 20 minutes. Formation of brick red precipitate confirmed the presence of reducing sugars.

Detection of glycosides

A minimum quantity of extract was hydrolysed with hydrochloric acid for few minutes on water bath and the hydrolysate was subjected to the following test**.**

Legals Test

To hydrolysate, 1 ml of the pyridine and few drops of sodium nitroprusside solution were added, then it was made alkaline with sodium hydroxide solution. Colour change showed the presence of glycosides.

Brontragers test

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this, equal quantity of dilute ammonia solution was added. Colour change in the layer showed the presence of glycosides.

Detection of proteins and amino acids

Small quantities of extracts were subjected to Millions, Biuret and Ninhydrin test.

Millions Test

The extract was treated with Millions reagent. Development of red colour indicated the presence of proteins.

Ninhydrin Test

The extract was treated with Ninhydrin reagent. Purple colour indicated the presence of proteins.

Biuret Test

To the extract, equal volume of 5% sodium hydroxide solution and 1% copper sulphate solution were added. A violet colour production indicated the presence of proteins.

Detection of fixed oils and fats

Spot Test

Small quantities of extract were placed between two filter papers. Oil strains were not produced and showed that the fats and fixed oils were absent in the extracts.

Saponification Test

Small parts of extract were treated with few drops of 0.5 N alcoholic potassium hydroxide along with 2 or 3 drops of phenolphthalein.

Later the mixture was heated on water bath for about 1-2 hours. There was no soap formation indicating the absence of fats and fixed oils in the extracts.

Detection of gums and mucilage

Ruthenium red Test

Small quantities of extracts were diluted with water and added with ruthenium red solution. There was no colour change which showed the absence of gums and mucilage.

Detection of alkaloids

Mayers test

1.2 ml solution of the extract was taken in a test tube, 0.2ml of diluted hydrochloric acid and 0.1ml of Mayer's reagent were added. Formation of yellowish buff coloured precipitate showed the positive test for alkaloids**.**

Dragendroffs test

2ml solution of the extract was treated with 0.1 ml diluted hydrochloric acid and 0.1 ml of Dragendroffs reagent in a test tube. Development of orange brown precipitate indicated the presence of alkaloids.

Wagners test

2 ml of the extract solution was treated with 0.2 ml of diluted hydrochloric acid and 0.1 ml Wagner's reagent. Development of reddish brown precipitate suggested the presence of alkaloids.

Hagers test

2 ml of solution of the extract was allowed to react with 0.2 ml diluted hydrochloric acid and 0.1 ml Hagers reagent. Formation of yellowish precipitate demonstrated the positive response for alkaloids**.**

Detection of phytosterols

Libermann-Buchard test

10mg of extract was dissolved in 1 ml of chloroform and 1 ml of acetic anhydride was followed by 2 ml of concentrated sulphuric acid.

Formation of reddish violet colour indicated the presence of steroids.

Salkowski test

When concentrated sulphuric acid was added to a chloroform solution of the extract (10 mg of extract in 1ml of chloroform), a reddish-blue colour was produced in the chloroform layer and green fluorescence in the acid layer which suggested the presence of phyto-steroids.

Noller test

5mg of the extract dissolved in 2 ml of 0.01 % of anhydrous stannic chloride in pure thionyl chloride. A pure colour formed, then changed to deep red after few minutes, indicated the presence of triterpenoid.

Detection of flavonoids

5 ml of the extract solution was hydrolysed with 10%v/v sulphuric acid and cooled. Then, it was extracted with diethyl ether and divided into three portions in three separate test tubes. 1 ml of diluted sodium carbonate, 1 ml of diluted ammonia solution were added to the first, second and third test tubes respectively. In each test tube, development of yellow colour demonstrated the presence of flavonoids.

Shinoda's test

The extract was dissolved in alcohol, to that one piece of magnesium followed by concentrated HCl were added drop wise and heated. Appearance of magenta colour showed the presence of flavonoids**.**

Detection of tannins-phenolic compounds

Potassium dichromate test

5ml of the extract was treated with 1 ml of 10 % aqueous potassium dichromate solution. Development of yellowish brown precipitate demonstrated the presence of tannins.

Lead acetate test

5 ml of extract was allowed to react with 1 ml of aqueous lead acetate solution and the yellow colour precipitate formation indicated the positive test for tannins.

Ferric chloride test

5 ml of extract was allowed to react with 1 ml of 5% ferric chloride solution. Formation of greenish black coloration demonstrated the presence of tannins.

Detection of saponins

Frothing test

1 ml solution of extract was diluted with distilled water to 20 ml and shaken in a graduated cylinder for 15 minutes. Formation of stable foam suggested the presence of Saponins.

Lead acetate test

1 ml of the extract solution was treated with 1% lead acetate solution. Development of white precipitate indicated the presence of Saponins.

Acute toxicity study

Albino mice of weight 25gm was procured from the Biomedical Research Unit and Laboratory Animal centre (BRULAC) of Saveetha University, Chennai (Reg.no865/ac/04/CPCSEA). The study was cleared by the institutional animal ethical committee. Three animals were taken and a 12-hour dark wake cycle was maintained with standard pellet food and water given *ad libitum.*

An acute toxicity study was conducted in accordance with OECD guideline 423. The animals were fasted prior to dosing, with water but not food given ad-libitum. The drug dosage was determined after weighing the fasted animals. A limit test was done at 2000 mg/kg as per previous studies and the drug was administered orally.

The animals were observed for signs of mortality and toxicity symptoms like changes in the colour of fur and eyes, mucous membrane and also other parameters like tremors, convulsions, salivation, diarrhoea, lethargy etc. at an interval of 15, 30, 60, 180 minutes, 6 hours, 24 hours and daily until 14th day.[13,14] On the 15th day, the animals were anesthetized using Ketamine and Xylazine and blood was collected for analysing haematological and biochemical parameters. The animals were later sacrificed by ketamine overdose.[15] The organs such as lung, liver, kidney, pancreas, spleen, brain were then removed, processed and sections of 5µ thickness were taken and stained by Haematoxylin and Eosin for histopathological studies.[4,16]

RESULTS

Preliminary Phytochemical Screening

Preliminary Phytochemical Screening of methanol extract of MP seeds are tabulated in the table 1. Table 1 shows the that the methanol extract of the MP seeds contains Carbohydrates, Proteins & amino acids, glycosides, alkaloids, phytosterols, flavonoids, saponins, tannins and phenolic compounds. However, fixed oil, fats, mucilage and gums were absent.

Acute toxicity studies

The animals were assessed for various wellness parameters which are tabulated in the table 2.

Table 1: Preliminary Phytochemical Screening of methanol extract of MP seeds

Test	Presence
Carbohydrates	+
Proteins & amino acids	+
Glycosides	+
Alkaloids	+
Phytosterols	+
Flavonoids	+
Saponins	+
Tannins & phenolic compounds	+
Fixed oils and fats	-
Gums and Mucilage	-

The assessment of various parameter shows that the state of wellness of all the animals was good and all the physiological processes were normal.

Haematological and biochemical parameters

The haematological parameters like Red Blood Corpuscle (RBC) count, White Blood corpuscle (WBC) count, Haemoglobin estimation (Hb), Packed Cell Volume (PCV), and biochemical parameters like blood glucose level, serum glutamate-pyruvate transaminase (SGPT), Serum glutamate oxaloacetate transaminase (SGOT), Alkaline phosphatase (ALP) levels were estimated and tabulated in the table 3 below.[9,17,18]

The table shows that the various parameters mentioned above were within the normal range.

Table 2: Assessment of animal wellness parameters

Parameter	0-15 min		15 min		30 min		60 min		120 min		180 min		24 hr		1st week		2nd week	
	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E
Skin & fur	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Mucous membrane	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Convulsions	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tremor	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Straub	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sedation	#	#	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Excitation	#	#	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Abnormal gait (rolling)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Jumps	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Motor incoordination	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of balance	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Writhes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pilorection	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stereotypies (head movements)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Head twitches	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Respiration	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aggressiveness	-	-	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
Fear	#	#	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Reactivity to touch	#	#	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Loss of righting reflex	#	#	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Akinesia	#	#	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Catalepsy	#	#	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of traction	#	#	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Loss of corneal reflex	#	#	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Analgesia	#	#	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Defeacation	#	#	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Salivation	#	#	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Lacrimation	#	#	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

C- Control Group E-Experimental Group; N-Normal, + /- Positive/Negative Response, # - Not Applicable

Table 3: Assessment of Haematological and biochemical parameters

Parameters	Values (mean ± St. Error of mean)
Haemoglobin (gm/dL)	12.32 ± 1.2
WBC(x10^3 cells/Cu.mm.)	8.2 ± 0.4
RBC (x10^6 cells/Cu.mm)	4.075 ±0.4
PCV	36.90% ± 0.03%
BLOOD GLUCOSE (mg/dL)	68.5 ± 8.0
SGPT (U/L)	47.25 ± 4.9
SGOT (U/L)	32.25 ± 6.9
ALP(U/L)	60.7 ± 11.72

Histopathology study

The histopathological studies of organs like liver, kidney, spleen, lung and pancreas were analysed with the help of a Pathologist (fig. 1,2,3,4,5). No abnormal cytoarchitecture, cell destruction, disorganisation and infiltration was noted.[18,19]

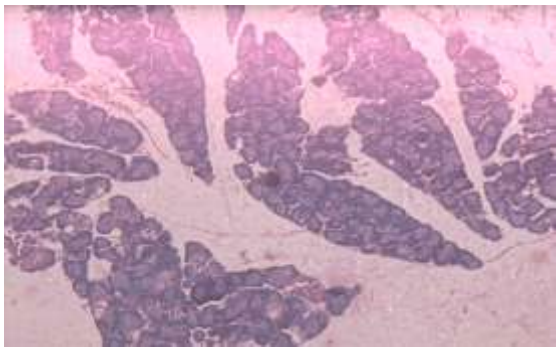

Figure 1: Section of pancreas (stained with H&E, x10)

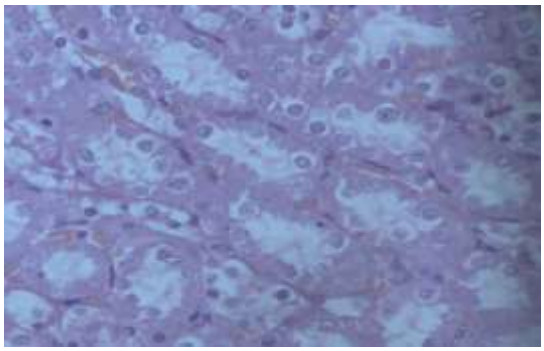

Figure 2: Section of Kidney (stained with H&E, x10)

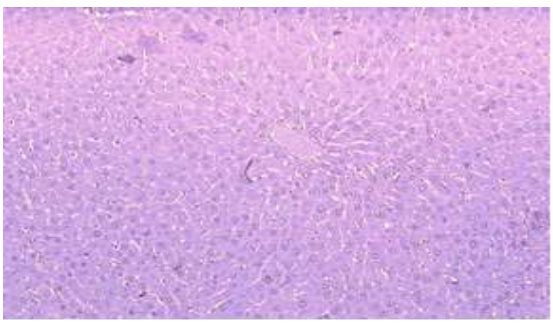

Figure 3: Section of liver (stained with H&E, x10)

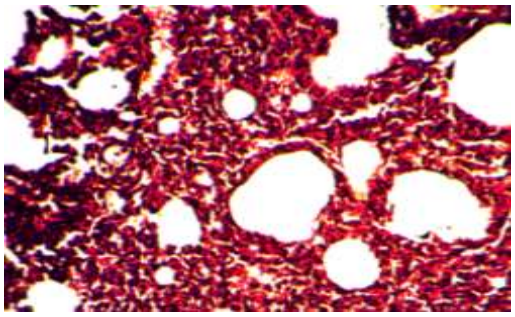

Figure 4: Section of lung parenchyma (stained with H&E, x10)

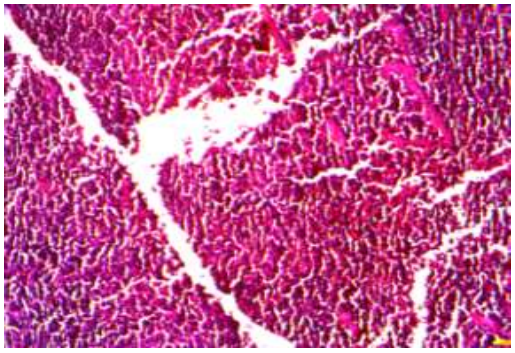

Figure 5: Section of spleen (stained with H&E, 10x)

The above figures 1,2,3,4,5 show the histopathological staining of pancreas, kidney, liver, lung and spleen. The images show that the cytoarchitecture of the cells were normal and there were no signs of cell destruction, disorganisation and infiltration.

DISCUSSION

The preliminary phytochemical study of methanol extract of Mucuna pruriens seeds are tabulated in table 1. The seeds of the Mucuna pruriens have been proposed to have a wide array of therapeutic benefits which is evident from its usage in various forms of alternative medicines. In our study, qualitative phytochemical analysis of the methanol extract of the seeds of Mucuna pruriens revealed the presence Carbohydrates, Proteins & amino acids, Glycosides, Alkaloids, Phytosterols, Flavonoids, Saponins, Tannins & Phenolic compounds. It is prudent to propose that it is their presence that may be the reason for the many of the proposed therapeutic effects of the seeds.[20] Our result is in consonance with various reports published earlier.[21,22]

The presence of flavonoids (mainly flavones) indicates that the extract can exhibit anti-lipidemic activity and hence can be used in the treatment of hypercholesterolemia.[23] Clinical studies demonstrate the beneficial role of saponins in reducing blood cholesterol. Saponins also play a pivotal role in immune system and have cancer reducing activity. Apart from this, it can also help in reducing dental caries and has anti-inflammatory activity.[10,24,25] But study by Deka Manalisha showed absence of Saponins but a similar study by Gavishiddappa A. Hadimani showed its presence.[26,21] According to studies by Leeds and walker (1982), carbohydrates

present in legume help in reducing cholesterol and also increases the blood glucose levels.[27,28] Recent studies by Nagamani shows that carbohydrates in the plant extracts has nutritive value and helps in conception, aids the process of maintaining pregnancy and promote normal delivery, probably as proposed by Murphy where, carbohydrates in the uterine endometrium plays a major role in the implantation of blastocyst.[29,30]

MP seed extract in our study was found to contain phytosterols which are plant sterols that are similar in structure to cholesterol but has anti-cholesterol effects by reducing total cholesterol and also low density lipoprotein (LDL).[31]

Tannins are polyphenols which has antioxidant properties as held by Dhanasekaran. Misra and Wager held the view that the amino acids present in extract (especially glutamic acid) also has a role to play in its antioxidant properties.[32] However they claim that the whole extract has more potent effects than of selective components.[9]

According to Fathima, the crude protein content of MP seeds is more when compared to pulse crops like black gram and green gram.[33]

The aphrodisiac property of MP seeds is being attributed to the major phytochemicals like alkaloids, flavonoids and saponins.[34]

The toxicity study shows that there was no mortality or toxicity symptoms noted in any of the animals in the group. The dose given was 2000mg/kg and the animal tolerated the dose well. Hence the LD 50 will be more than 2000 mg/kg.

This is concurring with the earlier studies by Gavishiddappa A. Hadimani, Deka Manalisha, Muhammad Mutasheem ul Hassan.[21,22,26] The analysis from table 2 shows that the wellness parameters of the animals were normal and no abnormal behaviour was noted as reported earlier by Deka Manalisha and Muhammad Mutasheem ul Hassan.[22,26]

The histopathological examination shows that the cytoarchiteture of the organs like liver, kidney, spleen, pancreas, lung appear normal and no disorganisation, infiltration or disturbances were noted.

CONCLUSION

The present study shows that the methanol extract of Mucuna pruriens seeds contains the important bioactive materials like Carbohydrates, Proteins & amino acids, Glycosides, Alkaloids, Phytosterols, Flavonoids, Saponins, Tannins & Phenolic compounds.

The drug is safe up to a dosage of 2000 mg/kg body weight as proven here by animal wellness parameters, biochemical parameters and also by histopathological studies.

Further studies are being done to improve our knowledge about the plant and its uses in various fields of medicine.

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