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Research Article

TOXICITY ASSESSMENT OF *MUCUNA PRURIENS* LINN. SEEDS

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ABSTRACT
Plants have been used as medicine for the treatment of diseases for thousands of years. These herbal remedies, although natural, can cause some serious damaging effects on the vital organs of the body due to inadequacy in standardization and safety regulations. *Mucuna pruriens* Linn. belongs to family Fabaceae and is used traditionally in various ailments. The present study reports the acute systematic toxicity and topical toxicity of methanolic extract of *Mucuna pruriens* (seeds) on albino mice and rabbits respectively. Test solution was injected intravenously into the tail as 1ml/20gm of the mice body weight. Observation was made immediately and after 1/2, 1, 4, 24, 48 and 72hours of drug administration. Mice showed normal activity till 72hours. The intracutaneous test is designed to evaluate local responses to the extracts of materials under test following intracutaneous injection into rabbits. The toxicological investigations of *Mucuna pruriens* with particular reference to intracutaneous toxicity in experimental animals displayed that it showed slight edema.
KEYWORDS: *Mucuna pruriens*, methanol extract, acute systematic toxicity, topical toxicity.

INTRODUCTION

Mucuna pruriens Linn. commonly known as velvet bean belongs to family Fabaceae. About 160 species of genus *Mucuna* are reported and distributed in the tropics. In Pakistan, it is represented by two species i.e. *Mucuna nigricans* Lour., and *Mucuna pruriens* Linn. *Mucuna pruriens* is a climbing herb, young branches densely pubescent, becoming glabrous. Leaf trifoliate, petiole 2-40cm long, leaflet 4.8-19cm long, 3.5-16.5cm broad. Inflorecence an axillary raceme, 15-30cm long, flowers single or 2-3 together. Fruit 5-6.3 cm long, not winged or plaited, with a longitudinal rib running the length of each valve, pubescent, hairs brown and irritating, 5-6 seeded[1] .All parts of *M. pruriens* possess valuable medicinal properties in traditional system of medicine and are used in bone fractures, cough, dog-bite, madness, pain, pleuritis, ring worm, scorpion sting, snake-bite, sores and syphilis. The seeds, pods and leaves are described as vermifuge[2]. The seeds are used for human food and animal feed in Nigeria[3] and one of the best sources of protein content [4]. They are used as an aphrodisiac, nerve tonic and are also useful in leucorrhoea, spermatorrhoea and scorpion sting. Leaves are applied to ulcers. The roots are said to be used in cholera, elephantiasis and also as a diuretic and purgative[2]. *Mucuna pruriens* is well known for producing itching. This property is attributed to the presence of 5-hydroxytryptamine (5-HT) in the hair on the pods[5]. The plant has been studied for various activities like anti-diabetic[6], aphrodisiac, anti-neoplastic, anti-epileptic, antimicrobial activities[7], learning and memory enhancement[8], antivenom[9], anthelmintic[10] and anti-inflammatory activities[11]. The seeds have been reported to be anti-diabetic[12], antifungal[13], anti-oxidant activity[14], hypotensive[15], hypocholesterolemic[16], hypothermic and antiparkinsonian activities[17].

Seeds contain alkaloids, glycosides, reducing sugars, saponins, tannins, terpenoids, calcium, phosphorus and potassium, polyphenolic substances, protease inhibitor, phytic acid and L-dopa[18].

Acute oral toxicity assessment of *Mucuna pruriens* has been published earlier[19] but there is no published report regarding its systemic study. The present study is further extension of toxicity studies. The acute systemic toxicity of *Mucuna pruriens* seeds methanolic extract was planned and carried out in Swiss albino mice and topical toxicity assessment by intracutaneous test in rabbits.

MATERIALS AND METHODS

Plant Sample Collection and Identification

The seeds of *Mucuna pruriens* were purchased from local market in Karachi. The plant material was identified for its authenticity and voucher specimen was kept in department of Pharmacognosy for future reference.

Plant Extraction

The seeds (2Kg) were crushed and soaked in methanol for 7 days at room temperature. The methanolic extract was evaporated under reduced pressure at 45°C.

Animals

Swiss male albino mice (17-23g) and albino rabbits (weight not less than 2.5 Kg) were purchased from Aga Khan University and Hospital animal house. All animals were kept under laboratory conditions of room temperature with 12/12h light and dark cycles and were allowed to free access to food and water *ad libitum*. The rabbits selected for tests were healthy thin skinned albino rabbits whose fur can be clipped closely and whose skin is free from mechanical irritation or trauma. The groups of animals were transferred in different cages and marked with their identification.

Materials

Electric shaver, mice trap, disposable syringes 1 ml, 0.2 µ membrane filters, filtration assembly, ethanol, normal saline solution.

Preparation of Test Sample

The sample (methanol extract) weighing 1.0 gram was dissolved in 200 ml water, to make the solution 0.5% (w/v). Before injection the solution was passed through 0.2 µ membrane filter. Each extract was agitated vigorously prior to withdrawal of Injection doses to ensure even distribution of the extract. It gave a concentration 5mg/ml.

Blank / Control Solution

Isotonic normal saline solution (0.9%) was used as blank solution.

Acute Systemic Toxicity Test

Acute systemic toxicity test was determined according to United State Pharmacopoeia[20]. Each group comprised of 5 albino mice. Healthy male mice were weighed on electric balance individually. Mice were fixed into the trap in a position that the tail was set free. The tails were disinfected with 70% ethanol. By using Insulin syringes 1.0 ml / 20 gm body weight, test solution was injected intravenously into the vein of the tail of each mouse. Time of the injection occupied about 15-30 seconds. Control Solution was also injected intravenously into the tail vein in the same manner as carried out for the test solution. Animals were observed immediately and at ½, 1, 4, 24, 48 and 72 hours after injection. Biological reactivity includes erection of hairs, skin diseases, difficulty in breathing, gross behavioral effects and any other abnormal activities were observed.

Topical Toxicity Test (Intracutaneous Test)

The biological reactivity of the methanolic extract of *Mucuna pruriens* was determined by intracutaneous test according to the United State Pharmacopoeia[20]. Local responses like erythema or edema have been determined for the seed extracts of *Mucuna pruriens* following intracutaneous injection into the albino rabbits. For each sample two rabbits were used. On the day of the test, the fur on the animals back on both sides of spinal cord was closely clipped and the loose hairs were removed by means of vacuum. Swabed the skin with diluted alcohol and five spots were marked 2.5cm away from the spinal column and 2cm away from each on both sides of spinal column. 0.2ml of test solution was injected intracutaneously at the spots of one side and similarly blank solution on the other side of spinal column. Mechanical irritations and trauma was avoided. Loose hairs were removed by means of vacuum. The same procedure was repeated on the other rabbits with test and blank solutions. The rabbits were examined immediately after injection, at ½, 1, 4, 24, 48 and 72 hours for any tissue reaction, like erythema or edema by swabbing the skin lightly with diluted alcohol to facilitate the reading. The average reaction of the injected sites was compared with the site of the blank solution. Observations were noted on numerical scale as shown in Table. I.

RESULTS AND DISCUSSION

Toxicity assessment of medicinal plants is necessary to evaluate their bioactivity for their safe therapeutic utilization. The present study is an attempt to investigate acute systemic toxicity and topical toxicity by intracutaneous test.

The acute systematic toxicity test and topical toxicity of methanolic extract of *Mucuna pruriens* seeds showed no mortality in experimental mice at 1ml / 20gm i.v., of the body weight. Also there were no changes in behavior (i.e., ataxia, hypo / hyperactivity) in any of the mouse, nor did show any variations in the general appearance during study. Table. II suggest the study protocol. As no mortality, no adverse changes in behavior as well as no abnormalities were detected during the course study so, seeds or seed extract of *Mucuna pruriens* prove to be safe and can be used for systematic action in the prevention and cure of diseases as use in traditional system of medicine.

The intracutaneous biological reactivity test (extract/blank) was conducted on two albino rabbits. Skin of the dorsal part along the vertebral column was selected for the intracutaneous administration of both *Mucuna pruriens* extracts and blank solution. The animals were examined for biological reactivity, which includes edema, erythema, necrosis followed by intracutaneous injection. Observations were made immediately after the administration of extract at ½, 1, 4, 24, 48 and 72 hours. The results of biological reactivity of seed extract of *Mucuna pruriens* are displayed (Table.III). Animals, which were injected with methanol extract after ½ hour have showed very slight edema (numerical value 1), after 1-4 hours showed same numerical value, after 24 hours showed slight edema (numerical value 2), which was remained same at 48th hour till the end of experiment (72 hours). The result of intracutaneous toxicity testing of the methanolic extract of seeds of *Mucuna pruriens* clearly predicted that extract produce slight edema (some sort of allergic reactions) throughout the study.

CONCLUSION

In view of the popularizing the use of medicinal plants in complementary and alternative medicine, it is necessary to carry out scientific research for standardization of medicinal plants with respect to their safety and toxicity assessment in laboratory animals to ascertain their safety for human use. The present research findings have clearly met the objectives of the study. In conclusion, the acute systematic toxicity shows that the methanolic extract of *Mucuna pruriens* could be regarded as safe in experimental mice for systemic action. Whereas same extract did not produce any sign of topical toxicity, except slight edema, when administered intracutaneously. However further detailed toxicological studies are required.

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Table NO.I EVALUATION OF SKIN REACTIONS.

Edema formation	Values
No edema	0
Very slight edema (barely perceptible)	1
slight edema (edges of area well defined by definite raising)	2
Moderate edema	3
Severe edema (raised more than 1mm and extending beyond the area of exposure)	4

Table NO.II STUDY PROTOCOL OF ACUTE SYSTEMIC TOXICITY OF *MUCUNA PRURIENS*.

Name of study	**Acute systematic toxicity of *Mucunapruriens* seeds**
Test material	Methanolic extract of *M.pruriens* seeds
Details of animal used	Healthy male swiss albino mice
Route of test drug administration	Intravenously into the tail
Dose of drug administration	1ml / 20gm of the mice body weight
Study duration	72 hours study period
Parameters observed	Mortality , sign of illness , injury , pain distress , allergic reactions, changes of outer appearance , difficulty in breathing , behavioral alterations (i.e., ataxia , hypoactivity , hyperactivity) and sedation

Table NO.III INTRACUTANEOUS TEST OF THE SEED EXTRACTS OF *MUCUA PRURIENS*.

TREATMENTS	**OBSERVATIONS**						
	Injection Immediately	**AFTER**					
		½ hour	**1 hour**	**4hour**	**24 hour**	**48 hour**	**72 hour**
Control	Normal 0	Normal 0	Normal 0	Normal 0	Normal 0	Normal 0	Normal 0
Methanolic extract	Very slight edema 1	Very slight edema 1	Very slight edema 1	Very slight edema 1	Slight edema 2	Slight edema 2	Slight edema 2

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