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Evaluation of Acute Oral Toxicity and Phytoconstituents of Methanolic Extract of *Mucuna pruriens*

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Abstract

The present research work was designed to identify the phytochemical constituents and acute oral toxicity of methanolic extract of seeds of *Mucuna pruriens*. Fresh mature seeds were shade dried at room temperature, coarse powdered and extracted with metahanol by Soxhlet's extraction method. Thereafter, the extract was concentrated using rotary flash evaporator to obtain semisolid crude extract with the yield of 09.534 %. *Mucuna pruriens* seed extract was investigated for the presence of phytochemical constituents and oral acute toxicity study. Preliminary phytochemical tests was conducted on test extract to detect the presence of phytochemicals by the standard methods described in the Pharmacognosy text book of Trease and Evans. The acute toxicity of test extract was determined in mice weighing between 20 – 25 g following fixed dose method of CPCSEA, OECD (Organization for Economic Cooperation and Development), guideline No. 420. The preliminary phytochemical evaluation of the *Mucuna pruriens* seed extract revealed the presence of steroids, alkaloids, tannins, carbohydrates, amino acid, resins and starch. In an acute toxicity studies, test extract of title plant did not cause any mortality of the animals at dose of 2000 mg/kg.

Key words: *Mucuna pruriens*, acute oral toxicity, phytochemical constituents.

INTRODUCTION

Nature always stands as a golden mark to exemplify the outstanding phenomenon of symbiosis. The biotic and abiotic elements of nature are all interdependent. The quest for long, healthy and happy life is as old as man himself. Nature has provided a complete storehouse of remedies to relieve the ailments of mankind. The consistent effects have resulted in many effective means of ensuring health care. The seers of Ayurveda were able to understand and record the various aspects regarding the drugs that even today are difficult to understand with modern available parameters [1, 2].

The medicinal plant products, which are derived from plant parts such as stem bark, leaves, fruits and seeds have been part of phytomedicine that produce a definite physiological action on the human body. The most important of these natural bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic compounds [3].

Itching bean Mucuna pruriens an underutilized legume species grown predominantly in Asia, Africa and in parts of America [4]. Mature seeds, seeds from unripe pods and young pods of itching bean, Mucuna pruriens are soaked and boiled/roasted and eaten as such or mixed with salt by the North-East Indian tribes; North-Western parts of Madhya Pradesh tribes; South Indian tribes [5-7]. To make this less- known legume palatable, tribal people follow a special processing method of continuous boiling and draining for about eight times until the boiled water changes from black to milky white. Consumption of improperly boiled seeds of itching bean is known to cause increase in body temperature and skin eruptions [8]. It is attributed to the presence of high levels of 3, 4- dihydroxy-L-phenylalanine, L-Dopa, the aromatic non-protein amino acid [9].

Hence, in the present study, the seeds of Mucuna prurienswere investigated and their chemical composition was investigated with a view to assess their phytochemical potential.

MATERIALS AND METHODS

Plant materials:

The seeds of Mucuna pruriens were procured locally after the seeds was authenticated by Dr. M B Mulimani Professor, Department of Botany. SB Arts & KCP Science College, Bijapur by the studies include organoleptic tests and macroscopic and microscopic observations. A voucher specimen has been deposited in our department.

Preparation of extract

Seeds were washed twice using tap water and then washed again in distilled water to remove the dust. The seeds were shade dried for 7–12 days at room temperature, until they were free from the moisture and then pulverized into coarse powder. The powdered material was extracted with methanol by Soxhlet's extraction method. Thereafter, the extract was concentrated using rotary flash evaporator to obtain semisolid crude extract. The percentage yield of the extract was found to be 09.534 %. The extract was stored in airtight container in refrigerator below 10^0C. Desired concentration of stock solution was prepared using distilled water for the following studies.

01. Preliminary phytochemical investigation.
02. Acute toxicity study in mice.

Preliminary phytochemical screening

Preliminary phytochemical tests were conducted on test extract to detect the presence of phytochemicals by following below mentioned the standard methods described in the Pharmacognosy text book of Trease and Evans.

1. **Test for Steroids** [11]

   Salkowski test: 2-3 drops of concentrated sulphuric acid was added to chloroform solution, shaken and allowed to stand, appearance of red color in lower layer indicates the presence of sterols.

   Liebermann-Burchard test: Extract was mixed with the chloroform and few drops of acetic anhydride and mixed well. Concentrated sulphuric acid was added from the sides of the test tube slowly until the ring appears; appearance of reddish brown ring indicates the presence of steroids.

2. **Test for Flavonoids** [12, 13]

   Shinoda test**:** To the extract a few fragments of magnesium ribbon and concentrated hydrochloric acid was added. Appearance of red to pink color after few minutes indicates the presence of Flavonoids.

   Lead acetate test: To the extract added few drops of aqueous basic lead acetate solution. Formation of yellow precipitate indicates presence of flavonoids**.**

   Alkaline reagent test/ NaOH test: few drops of sodium hydroxide solution was added to extract. Intense yellow color disappeared after adding dilute HCl which indicates the presence of flavonoids.

3. **Test for Alkaloids**: The extract was basified with ammonia and extracted with chloroform. The chloroform solution was acidified with dilute hydrochloric acid, shaken well and filtered. The filtrate was used for testing the alkaloids.

   Hager's test*:* The filtrate was treated with few drops of Hager's reagent. Formation of yellow precipitate indicates the presence of alkaloids [14].

   Wagner's test (Iodine in Potassium iodide): The acid layer was treated with few drops of Wagner's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

   Mayer's test (Potassium Mercuric Iodine solution): The acid layer was treated with few drops of Mayer's reagent. Formation of creamy white precipitate indicates the presence of alkaloids.

   Dragendorff's reagent (Potassium Bismuth Iodide): The acid layer was treated with few drops of Dragendorff's reagent. Formation of reddish brown precipitate indicates the presence of alkaloids.

4. **Test for Tannins**[15]

   Gelatin test: To the extracts of the drug added 1% solution of gelatin containing 10% sodium chloride. Formation of white precipitate indicates the presence of tannins.

   Ferric chloride test: To extracts few drops of 1% neutral ferric chloride solution were added, formation of blackish blue color indicates the presence of tannins.

5. **Test for Saponins** [14, 16]

   Foam test**:** Small amount of extract of the drug was shaken with little quantity of water, if foam produced persists for 10 minutes; it indicates the presence of saponins.

   Froth test**:** To 5 ml of extract of the drug added single drop of sodium bicarbonate solution. Shaken the mixture vigorously and left for 3 minutes. Formation of honey comb like froth indicates presence of saponins.

6. **Test for Carbohydrates:** Small amount of extracts of the drug were dissolved in little quantity of distilled water and filtered separately. The filtrates were used to test presence of carbohydrates.

   Molisch's test: The filtrate of the drug was treated with Molisch reagent and concentrated sulphuric acid was added from the sides of the test tube to form a layer. A reddish violet ring shows the presence of carbohydrates.

   Benedicts test: to the filtrate added 2 ml Benedict's reagent and boiled in water bath. Formation of Green or reddish brown precipitate indicates presence of carbohydrates.

   Fehlings test: Filtrates were hydrolyzed with dilute hydrochloric acid, neutralized with alkali and heated with equal amount of Fehling's A and B solutions. Formation of green to yellow to red precipitate indicated the presence of reducing sugars.

7. **Test for Amino acid/ Protein**

   Ninhydrin test: Heated the 3 ml of extract of the drug and 3 drops of ninhydrin solution in boiling water bath for 10 minutes. Appearance of purple color shows the presence of amino acids.

   Biuret test: To 3 ml of extract of the drug added 4% NaOH and few drops of 1% copper sulphate solution. Formation of violet color confirms the presence of protein.

   Millon's reagent test: Mixed the extract with millon's reagent. Formation of brick red precipitate indicates the presence of protein.

8. **Test for Resins:** Dissolved the extract in acetone and pour the solution in to distilled water. Turbidity indicates the presence of resins [10].

9. **Test for starch:** dissolved 0.015 gm of iodine and 0.075 gm of potassium iodide in 5 ml of distilled water and add 2-3 ml of an aqueous extract of drug, blue color is produced.

Experimental animals

Female albino Swiss mice (20 - 25 g) were used in the experiments. Animals were procured from central animal house, BLDEU Sri B M Patil Medical College, Bijapur, India. After randomization into various groups and before initiation of experiment, the animals were acclimatized for a period of 10 days. Animals were housed in polypropylene cages and maintained under standard environmental conditions such as temperature (26 ± 2^0C), relative humidity (45 - 55%) and 12 hr. dark/light cycle. The animals were fed with rodent pellet diet and water *ad libitum*. The study protocol was approved from the Institutional Animal Ethics Committee (IAEC) before commencement of experiment.

Determination of acute toxicity (LD_{50})

The acute toxicity of test extract was determined in mice weighing between 20 - 25g following fixed dose method of CPCSEA, OECD (Organization for Economic Cooperation and Development), and guideline No. 420; (Annexure-2d) [17].

RESULTS

Preliminary phytochemical screening

Preliminary Phytochemical screening of methanolic extract of seeds of Mucuna pruriens revealed the presence of different kind of phytochemical components that are summarized in table 1

Table 1: Preliminary phytochemical screening of Methanolic Mucuna pruriens seed extract

S. No	Phytochemical	Test	Result
1	Test for Steroids	Salkowski test:	Present
		Liebermann-Burchard test	Present
2	Test for Flavonoids	Shinoda test	Absent
		Lead acetate test	Absent
		Alkaline reagent test/ NaOH test	Absent
3	Test for Alkaloids	Hager's test	Present
		Wagner's test	Present
		Mayer's test	Present
		Dragendorff's reagent	Present
4	Test for Tannins	Gelatin test	Present
		Ferric chloride test	Present
5	Test for Saponins	Foam test	Absent
		Froth test	Absent
6	Test for Carbohydrates	Molisch's test	Present
		Benedicts test	Present
		Fehlings test	Present
7	Test for Amino acid/ Protein	Ninhydrin test	Present
		Biuret test	Present
		Millon's reagent test	Present
8	Test for Resins		Present
9	Test for starch		Present

Determination of acute toxicity (LD_{50})

In an acute toxicity studies, test extract of title plant did not cause any mortality of the animals at dose of 2000 mg/kg.

DISCUSSION:

These results obtained in the present study are in good consonance with the earlier reports of *Mucuna pruriens*. Steroids, alkaloids, tannins, carbohydrates, amino acid and resins were present in methanolic extract of seeds of *Mucuna pruriens*. The medicinal values of the seeds may be related to their constituent phytochemicals. According to Varadarajan et al., the secondary metabolites (phytochemicals) and other chemical constituents of medicinal plants account for their medicinal value [18]. For example, saponins are glycosides of both triterpene and steroids having hypotensive and cardiodepressant properties, while anthraquinones posses' astringent, purgative, anti-inflammatory, moderate antitumor, and bactericidal effects [19&20].

In the present study methanolic extract of seeds of *Mucuna pruriens* have significant amount of alkaloids and those are responsible for most varied type of pharmacological actions and have the effect on central nervous system probably may help in relieving the causes of infertility and maintenance of pregnancy with overall influence on the body and fetus. Presence of steroids indicates that these drugs may have influence on endocrine system. As steroids are precursors for synthesizing sex hormones especially progesterone and estrogen which are basic factors for infertility. Tannins were positive in Mucuna pruriens and contributory to overcome the possible haemorrhagic or other discharges of yoni andhelps conception. Mucuna pruriens indicates presence of both carbohydrates and starch this indicate they are polysaccharides. They are nutritive, help for conception and maintain pregnancy and promote normal delivery. Because of presence of resins in some Mucuna pruriens it has been used as antibacterial and anti-fungal. There are conditions in infertility which are due in inflammatory lesions which may be overcome by presence of resins. Presence of proteins in Mucuna pruriens has been described by Fathima et al. Mucuna pruriens contain higher crude protein when compared with commonly consumed pulse crops such as black gram, green gram, pigeon pea, chick pea and cow pea [21-22].

CONCLUSION

In conclusion, the findings of the present study suggest that seeds extract of the Mucuna pruriens possesses steroids, alkaloids, tannins, carbohydrates, amino acid, resins and starch. In an acute toxicity studies, test extract of title plant did not cause any mortality of the animals at dose of 2000 mg/kg.

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