

EVALUATION OF *MUCUNA PRURIENS* SEED EXTRACT FOR ITS ACUTE ORAL TOXICITY IN ALBINO RATSGANGADHARA SWAMY¹, SURESH R RAO^{1*}, RAJENDRA HOLLA²¹Department of Anatomy, Subbaiah Institute of Medical Sciences and Research Center, Shivamogga, Karnataka, India. ²Department of Pharmacology, K. S. Hegde Medical Academy, Mangalore, Karnataka, India. Email: s4chavan@yahoo.co.in

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ABSTRACT

Objectives: The present study was carried out to evaluate the hydroalcoholic extract of *Mucuna pruriens* (HAMP) seeds for its acute oral toxicity in albino rats.

Methods: Acute oral toxicity of MP seed extract was assessed in albino rats with three different doses of the extract with 175, 550, and 2000 mg/kg body weight. Body weight, mortality, and clinical signs were recorded on 0 (before administration), 7th, and 14th days. Rats were sacrificed after day 14 and observed for any histological changes in the brain, heart, liver, and kidney tissues. Rats were normal up to 1 h and exhibited dullness and piloerection after 1 h which continued up to 2–4 h of observation period on day 0 of administration. All animals appeared normal from day 1 to throughout the experimental procedure.

Results: No significant changes in the histological structure of the liver, kidney, and heart were noticed except mild congestion and hydropic changes only in liver tissue seen for 2000 mg/kg body weight of HAMP seeds. The seed extract of MP is non-toxic to rats and did not show any mortality nor the behavioral changes. In addition, it showed an increase in the body weight with the administration up to 2000 mg/kg body weight.

Conclusion: MP seed extract signified as neurosuppressant, and the drug can be used in the treatment of neurological disorders characterized by hyperactivity of the neurons. The present data could provide adequate confirmation of the safety of MP for further experimental studies on a standardized formulation of the seeds extract.

Keywords: *Mucuna pruriens* seeds, Acute oral toxicity, OECD guidelines 425, Median lethal dose, Neurosuppressant.

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INTRODUCTION

Mucuna pruriens (MP) commonly called velvet bean is a tropical legume indigenous tree to Africa and tropical Asia. As it is one of the best sources of protein content in many African countries, it is used as food for humans and also the animal feed [1]. MP seed pods are covered with hairs which are rich in 5-Hydroxy tryptamine (5-HT) & if they come in touch with skin that causes severe itching [2,3]. Its seeds contain many micronutrients including amino acids, zinc, selenium, carbohydrates [4], and various plant alkaloids [5]. In addition to these ingredients, the seeds' main ingredient is L-DOPA [6]. In Ayurveda, Parkinson's disease (PD) is referred as Kampavata, which is one among eighty Vathaja diseases (Astha Mahagathavatarogas), and there are a sufficient number of references explaining in detail for the PD for its cause, prognosis, and its treatment using many medicinal formulations with main ingredient as the MP seeds [7,8-10]. Numerous research works have been proven that MP seeds contain L-DOPA [1,11-13], which is a just precursor to the neurotransmitter dopamine during its synthesis, which is found in the nigrostriatal pathway of the basal nuclei of the midbrain [14].

According to Akhtar *et al.*, the ethanolic extract of seeds of MP possesses anti-cataleptic and antiepileptic effect in albino rats, and dopamine and serotonin may have a role in such activity [15]. Apart from this, it is reported from this plant that it is also having antiurolithiatic [14], antidiabetic [15-17], anticancer, and antioxidant properties [18]. However, if consumed in large quantities of crude MP seeds as food, it is poisonous to mammals [19,20]. This indicates that there is some toxicity related to MP.

As per the OECD guidelines before the clinical trial on humans for any drug, it should be tested on animals to define its median oral toxicity

(lethal dose [LD₅₀]) values and its effective/therapeutic dose. The name LD₅₀ is an abbreviation for "median LD₅₀ 50%." It is the amount of the substance required (usually per body weight) to kill 50% of the test population. LD₅₀ is the amount of a drug, given oral all at once, which causes the death in >50% (one half) of experimental animals in a group [21]. The LD₅₀ is one way to measure the short-term poisoning potential (acute toxicity) of a material. Rats and mice are most routinely used for the experiment by the toxicologist. It is usually expressed as the amount of chemical administered (e.g., mg) per 100 g (for smaller animals) or per kilogram (for bigger test subjects) of the body weight of the test animal. The LD₅₀ can be found for any route of entry or administration, but dermal (applied to the skin) and oral (given by the mouth) administration methods are the most common [22-24].

MATERIALS AND METHODS**Plant material**

The seeds of MP were collected from the Sri Dharmastala Ayurveda Medical College and Research Center, Udupi, Karnataka, India. The plant material was stored in ambient conditions for further study.

Preparation of extract

The MP seeds were dried in shade and powdered in our research laboratory with the help of pulverizer. The hydroalcoholic extract of MP (HAMP) seeds were prepared by soaking 500 g of powdered seeds of MP in 2 liters of 50% ethanol and 50% cold distilled water for 24 h, filtered, and concentrated by evaporating on water bath till free from water. The extract has been stored in airtight container under normal temperature [10].

Experimental animals

This study was performed in a CPCSEA approved laboratory under registration number 115/1999/CPCSEA following all ethical practices

as laid down in the guidelines for animal care. This study has been approved by the Institutional Animal Ethics Committee (IAEC) KSHEMA/IAEC/08/2017. The study procedure described in this study met the requirements of OECD guidelines for testing of chemicals, number 425, "acute oral toxicity-acute toxic class method."

Female albino rats (9–11 weeks, weighing between 200 and 250 g) were used for the experiment. All animals were maintained under standard laboratory conditions, with a constant 12 h light/dark cycle and controlled temperature (25±2°C) with access to drinking water and pellet diet *ad libitum* [25].

Chemicals

The solvents and chemicals required carboxymethyl cellulose (CMC) as a suspending agent and distilled water as a solvent.

Methodology

Female albino rats were selected based on their days of acclimatization. The rats were divided into three groups, namely Group A, Group B, and Group C, with each group having six rats (n=6). They were kept fasting for overnight (but with the free access to water). On the test day 0, the rats in each group had received a single dose of the plant extract by oral gavage method dosages at 175, 550, and 2000 mg/kg body weight, respectively. Approximately after 17 h of fasting but with free access to water, the animals were continued with proper diet [5-8].

Calculation for the preparation of the stock solution

- Group A: 175 mg of MP is mixed with 50 mg of CMC and the mixture is dissolved in 10 ml distilled water to get the concentration of 17.5 mg/ml.
- Group B: 550 mg of MP is mixed with 50 mg of CMC and the mixture is dissolved in 10 ml distilled water to get the concentration of 55 mg/ml.
- Group C: 2000 mg of MP is mixed with 50 mg of CMC and the mixture is dissolved in 10 ml distilled water to get the concentration of 200 mg/ml.

The animals were observed for the acclimatization before the oral gavage of the drug and also daily during the test period for clinical signs such as sedative effect, decreased locomotor activity, breathlessness, and mortality/viability and were recorded during first 30 min and at approximately with the duration of 1, 2, 3, and 4 h after administration of test drug on day 0 and daily twice during days 1–14 [24,25]. Body weights of all the rats were recorded on test day 0 (before administration) and also on test days 7 and 14.

At the end of the 14th day of observation period, the animals were deeply anesthetized with ether. All the animals were observed for any gross/macrosopic pathological changes, and the brain, liver, heart, and kidneys from the representative groups of animals were removed and processed for the histological studies as follows [26].

Table 1a: Mortality and clinical signs observed with *Mucuna pruriens* seed extract (175 mg/kg body weight) in rats over a period of 14 days

Animal ID No.	Test days					1	2	3	4	5	6	7	8	9	10	11	12	13	14
	0*																		
	30 min	1 h	2 h	3 h	4 h														
A1	N	D	D	D	D	D	N	N	N	N	N	N	N	N	N	N	N	N	N
			P	P	P														
A2	N	D	D	D	D	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			P	P	P														
A3	N	D	D	D	D	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			P	P	P														
A4	N	D	D	D	D	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			P	P	P														
A5	N	D	D	D	D	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			P	P	P														
A6	N	D	D	D	D	D	N	N	N	N	N	N	N	N	N	N	N	N	N
			P	P	P														

N: Normal, D: Dullness, P: Piloerection

Table 1b: Mortality and clinical signs observed with *Mucuna pruriens* seed extract (550 mg/Kg body weight) in rats over a period of 14 days

Animal ID No.	Test days					1	2	3	4	5	6	7	8	9	10	11	12	13	14
	0*																		
	30 min	1 h	2 h	3 h	4 h														
B1	N	D	D	D	D	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			P	P	P														
B2	N	D	D	D	D	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			P	P	P														
B3	N	D	D	D	D	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			P	P	P														
B4	N	D	D	D	D	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			P	P	P														
B5	N	D	D	D	D	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			P	P	P														
B6	N	D	D	D	D	N	N	N	N	N	N	N	N	N	N	N	N	N	N
			P	P	P														

N: Normal, D: Dullness, P: Piloerection

Table 1c: Mortality and clinical signs observed with *Mucuna pruriens* seed extract (2000 mg/kg body weight) in rats over a period of 14 days

Animal ID No.	Test days																		
	0					1	2	3	4	5	6	7	8	9	10	11	12	13	14
	30 min	1 h	2 h	3 h	4 h														
C1	D	D	D	D	D	D	N	N	N	N	N	N	N	N	N	N	N	N	N
		P	P	P	P														
C2	D	D	D	D	D	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		P	P	P	P														
C3	D	D	D	D	D	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		P	P	P	P														
C4	D	D	D	D	D	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		P	P	P	P														
C5	D	D	D	D	D	N	N	N	N	N	N	N	N	N	N	N	N	N	N
		P	P	P	P														
C6	D	D	D	D	D	D	N	N	N	N	N	N	N	N	N	N	N	N	N
			P	P	P														

N: Normal, D: Dullness, P: Piloerection

Table 2a: Physical observations in experimental rats with *Mucuna pruriens* seed extract (175 mg/kg body weight) at terminal sacrifice.

Animal ID No.	Physical observations in experimental rats
A1	No abnormality detected
A2	No abnormality detected
A3	No abnormality detected
A4	No abnormality detected
A5	No abnormality detected
A6	No abnormality detected

Table 2b: Physical observations in experimental rats with *Mucuna pruriens* seed extract (550 mg/kg body weight) at terminal sacrifice

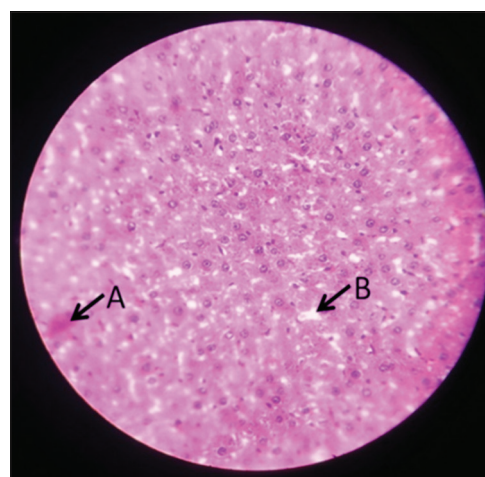
Animal ID No.	Physical observations in experimental rats
B1	No abnormality detected
B2	No abnormality detected
B3	No abnormality detected
B4	No abnormality detected
B5	No abnormality detected
B6	No abnormality detected

Table 2c: Physical observations in experimental rats with *Mucuna pruriens* seed extract (2000 mg/kg body weight) at terminal sacrifice

Animal ID No.	Physical observations in experimental rats
C1	No abnormality detected
C2	No abnormality detected
C3	No abnormality detected
C4	No abnormality detected
C5	No abnormality detected
C6	No abnormality detected

Procedure

The animals were deeply anesthetized with ether and fixed on a dissection board, and its chest cavity was opened to expose the heart. About 15 ml of 0.9% saline was perfused through the left ventricle at the rate of 1 ml/min. This was followed by perfusion with 10% formalin, about 250 ml/adult rats, at the same rate. The animals were decapitated, and 5–6 mm thick coronal section of the brain with the cerebral cortex, midbrain, liver, kidneys, and heart were removed and

**Fig. 1: Histological picture of the liver (magnification, ×40) at the dose 2000 mg/kg body weight of HAMP. Arrow mark A - shows mild congestion and B - shows hydrophic changes**

kept in 10% formalin for 24 h (post-fixation). Paraffin blocks were made given below.

Statistical analysis

Statistical analysis is performed using Student's *t*-test and one-way ANOVA where ever it is applicable.

RESULTS AND DISCUSSION

Oral administration of HAMP at three different doses for 14 days in albino rats did not produce any significant toxicity symptoms in rats, including the highest dose tested at 2000 mg/kg body weight. No obvious clinical signs were found in any groups at initial 30 min of observation.

Changes in the clinical symptoms such as dullness and piloerection were noted after 1 h which continued up to 4 h of observation period on 0 day. Rats of all the three doses appeared normal and did not show signs of toxicity in their skin, fur, eyes, diarrhea, and behavior from day 1 and continued to remain normal throughout the experimental period (Table 1a-c).

A brief period of dullness on day 0 indicates a possible neurosuppression role of the drug extract. We did not find any significant changes in the mortality rate in any groups exposed to orally administered HAMP Albino rats.

Table 3: Changes in body weight of experimental rats with *Mucuna pruriens* seed extract (175, 550, and 2000 mg/kg body weight) at 0, 7, and 14 days

Groups (mg/kg body weight)	Number of animals (n)	0 day	7 th day	14 th day
175	6	226.77±6.036	240.35±6.354*	251.00±6.392*
550	6	205.97±1.458	214.03±1.492*	220.55±1.479**
2000	6	212.43±0.6407	222.01±1.085*	233.75±1.917***

Values are expressed as mean±SE. *p values were considered significant using one-way ANOVA. *p<0.05, ANOVA significance F=3.761 for 175 mg/kg body weight, **p<0.01, ANOVA significance F=24.48 for 550 mg/kg body weight and ***p<0.001, ANOVA significance F=64.954 for 2000 mg/kg body weight

No gross abnormalities were observed in any animals until the terminal sacrifice period (Table 2a-c).

A significant increase in the body weight was observed in all the rats subjected for the study by day 14 as compared to 0 days of the experimental animals (Table 3).

Light microscopic examination of sections of various organs such as the liver, heart, and kidney of the drug-administered groups showed a normal cytoarchitecture and absence of any gross pathological lesions, in all the three groups except mild congestion and hydrophilic changes found in the liver at the dose 2000 mg/kg body weight of HAMP drug-administered rats when compared with normal (Fig. 1).

Above results indicate that, after oral administration of the HAMP seed extract, rats appeared dull for a brief period (on test day 0), after which they became normal throughout the experimental procedure (until 14 days). Interestingly, no mortality was observed in the rats. This temporary dullness indicates a possible role of the neurosuppressive effect of the extract. The above experiment has recorded a lethal dose of the HAMP which is more than 2000 mg/Kg body weight, and the same values can be practiced for the further studies to define its effective dose and the therapeutic index, so that it can be safely used as a drug in the treatment of neurological disorders characterized by the hyperactivity of neurons. Moreover, it was also observed that all the rats had gained body weight by the end of the experimental procedure. This establishes that the L-DOPA-enriched extract of MP is non-cytotoxic to the rats. Based on the results obtained, the LD₅₀ of HAMP after single oral administration to female rats, observed over a period of 14 days, is >2000 mg/kg body weight. Sardjono *et al.* also showed non-toxic effect of MP seed extracts, but it was carried out using ethanol extract, which supports our study [27].

CONCLUSION

MP seed extract was evaluated for its acute oral toxicity in albino rats. It was found that HAMP at high dosage (2000 mg/Kg body weight) was non-toxic to the rats and it too aided in bringing in the body weight. In addition, a brief period of dullness indicates a possible role of the extract in neurosuppression as well. Thus, it can be used safely as a drug in the treatment of neurological disorders characterized by the hyperactivity of neurons. The results of this study also collectively specify that oral administration of HAMP is not connected with any toxicological significant effects and the data could provide satisfactory preclinical evidence of safety to launch a clinical trial on a standardized formulation of the plant extracts. Based on the study, we are able to estimate the LD₅₀ of the test drug, which can be used safely for further experimental studies on the rats, and to calculate its therapeutic dose and also to know its anti-Parkinsonism effect in rotenone-induced Parkinsonism-diseased rats.

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AUTHORS' CONTRIBUTIONS

The first author designed and worked on the experiment, the second author collected the review of literature and also planned for the

experiment, and the third author helped in planning and statistical analysis of the work. All the three authors equally contributed for the overall study.

CONFLICTS OF INTEREST

The authors do not have any conflict of interest on the publication of this manuscript.

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