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Acute oral toxicity study in rats with *Mucuna pruriens* seed extract

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ABSTRACT

Acute oral toxicity of Mucuna pruriens seeds extract was assessed in Wistar rats with a single high dose of the extract at 2000 mg/Kg body weight. Mortality/viability and clinical signs were recorded on test day 0 (prior to administration), test days 7, 14 and at death. Rats showed dullness during first 30 min and 1 h and piloerection at 2, 3, 4 h of observation period on day 0 of administration. All animals appeared normal from day one to throughout the experimental procedure. The seed extract is non toxic to rats and helped in weight gain with LD_{50}> 2000 mg/Kg body weight. Additionally, it acted as neuro- suppressant. Thus it can be used as a drug in treatment of neurological disorders characterized by hyper activity of the neurons. To summarize, the results of this study collectively specify that oral administration of Mucuna pruriens is not connected with any toxicologically 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.

Keywords: *Mucuna pruriens* seeds, Acute Oral Toxicity, OECD Guidelines 423, Lethality (LD50), Neuro suppressant

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INTRODUCTION

Mucuna pruriens (L.) DC. (Commonly called velvet bean) is a tropical legume indigenous to Africa and tropical Asia.Its seed pods are covered by hairs that cause a severe irritation if they come in contact with skin [1-2]. The chemical compounds responsible for the itch are mucunain and serotonin [3]. The plant and its extracts have been long used by tribes as a toxin antagonist for various snakebites including Cobra, Pit Viper and Krait [4-6]. The seeds of *Mucuna pruriens* have been used in traditional Ayurvedic Indian medicine for treating diseases including Parkinson's disease [7-8]. The mature seeds of the plant contain about 3.1–6.1% L-DOPA [1, 9-11], with trace amounts of serotonin, nicotine, DMT-n-oxide, bufotenine, 5-MeO-DMT-n-oxide, and beta-carboline.Due to presence of these chemicals it has potential psychedelic effects and is being used for treatment of Parkinson's disease [12-14]. The beans have also been used as a powerful aphrodisiac in Ayurveda [15-17] and for the treatment of various nervous disorders and also arthritis [18].

The ethanolic extract of leaves of *Mucuna pruriens* possesses anticataleptic and antiepileptic effect in albino rats. Dopamine and serotonin may have a role in such activity [19]. Apart from being antiurolithiatic[20], the anti-diabetic, anti cancer and anti-oxidant properties have also been reported from this plant [2, 21-24].

If consumed in large quantities as food, crude *Mucuna pruriens* is poisonous to mammals [25]. This indicate that toxicity is the main issue related to *Mucuna pruriens* therefore, in the present article, we prepared *Mucuna pruriens* seed extract and assessed its oral toxicity in the rats.

MATERIALS AND METHODS

Plant Material:
The seeds of *Mucuna pruriens* were gifted by Patanjali Ayurveda Ltd, Haridwar, India. The plant material was stored in ambient conditions for further study.

Preparation of Extract:
The powdered seeds of *Mucuna pruriens* (100 gm)were subjected to extraction using Methanol: Water (60:40). The extract was evaporated to dryness in a rotary flash evaporator at a temperature not exceeding 60°C, and then stored in air tight container. The concentrate was passed through HCl treated Amber litecation resin. The resin was washed thoroughly with Ammonium hydroxide and concentrated to obtain L-DOPA enriched extract.

Experimental Animals:
Female Wistar rats (9-11 weeks, weighing between 170-200 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 (22 ± 2 °C)with access to drinking water and pellet diet (Lipton India Ltd.) *ad libitum*.

Chemicals:
The solvents and chemicals required for the study were purchase from Sigma-Aldrich, India.

Methodology:
This study was performed in a CPCSEA approved laboratory under registration number 1010/bc/06/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) 1410/2009. The study procedure described in this study meet the requirements of OECD guidelines for testing of chemicals, number 423, "Acute Oral Toxicity-Acute Toxic Class Method."

Test Procedure followed:
Female Wistar rats were divided into two groups, based on the days of acclimatization (5 days for Group I and 7 days for Group II). The animals received a single dose of the extract by oral administration at a high dosage of 2000 mg/Kg body weight, after being fasted for approximately 17 h but with free access to water. The food was provided again approximately 3 h after providing the dose. The extract was formulated in distilled water at a concentration of 200 mg/ml and the dose volume of 10 ml/Kg was administered. The animals were observed daily during the acclimatization period, mortality/viability and clinical signs were recorded. All animals were observed for clinical signs during first 30 min and at approx. 1, 2, 3 and 4 h after administration on test day 0 and once daily during test days 1-14. Mortality/viability was recorded during first 30 min and at approx 1, 2, 3 and 4 h after administration on test day 0 and twice daily during days 1-14 (at least once on day of sacrifice). Body weights were recorded on test day 0 (prior to administration), test days 7, 14 and at death. All the animals were sacrificed at the end of the observation period by CO_2 in euthanasia chamber and discarded after gross/ macroscopic pathological changes were observed and recorded.

RESULTS AND DISCUSSION

All the animals of Group I dosed at 2000 mg/kg body weight showed dullness during first 30 min and 1 h after administration and then dullness and piloerection at 2, 3, 4 h of observation period on day 0 of administration. All animals show dullness on day 1 after administration. All animals appeared normal from day 2 to throughout the experimental procedure. While in Group II animals showed dullness during first 30 min after administration and then dullness and piloerection at 1, 2, 3 h and only dullness at 4 h of observation period on day 0 of administration. All animals appeared normal from day 1 to throughout the experimental procedure (**Table 1**). A brief period of dullness indicates a possible role of the extract in neuro-suppression as well.

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

Group	Animal ID no.	Test days																		
		0*					1	2	3	4	5	6	7	8	9	10	11	12	13	14
		0.5	1	2	3	4														
I	1	D	DP	DP	DP	DP	D	N	N	N	N	N	N	N	N	N	N	N	N	N
	2	D	DP	DP	DP	DP	D	N	N	N	N	N	N	N	N	N	N	N	N	N
	3	D	DP	DP	DP	DP	N	N	N	N	N	N	N	N	N	N	N	N	N	N
II	4	D	DP	DP	DP	D	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	5	D	DP	DP	DP	D	N	N	N	N	N	N	N	N	N	N	N	N	N	N
	6	D	DP	DP	DP	D	N	N	N	N	N	N	N	N	N	N	N	N	N	N

N=Normal, D=Dullness, P= Piloerection

**Examinations were performed within the first 0.5 h and at approximately 1, 2, 3 and 4 h after treatment on test day 0*

Table 2 Changes in body weight of experimental rats with *Mucuna pruriens* seed extract (2000 mg/Kg body weight) at 0, 7, 14 days

Group	Animal ID number	Weight (g)		
		Test day 0*	Test day 7	Test day 14
I	1	170.3±2.5	189.8± 0.8	200.5± 0.7
	2	179.5± 1.2	193.9± 1.9	199.6± 1.8
	3	173.7± 0.8	184.1± 3.0	193.8± 1.1
II	4	194.1± 1.6	212.0± 1.4	220.2± 2.3
	5	181.2± 2.9	189.3± 0.3	197.9± 1.4
	6	190.0± 0.3	200.9± 3.1	208.2± 0.9

0 (test day), weight noted same day after the treatment*

Table 3 Physical observations in experimental rats with *Mucuna pruriens* seed extract (2000 mg/Kg body weight) at terminal sacrifice

Dose (mg/Kg body weight)	Group	Animal ID no.	Physical observations in experimental rats
2000	I	1	No Abnormality Detected
		2	Hydrometra
		3	No Abnormality Detected
	II	4	No Abnormality Detected
		5	No Abnormality Detected
		6	No Abnormality Detected

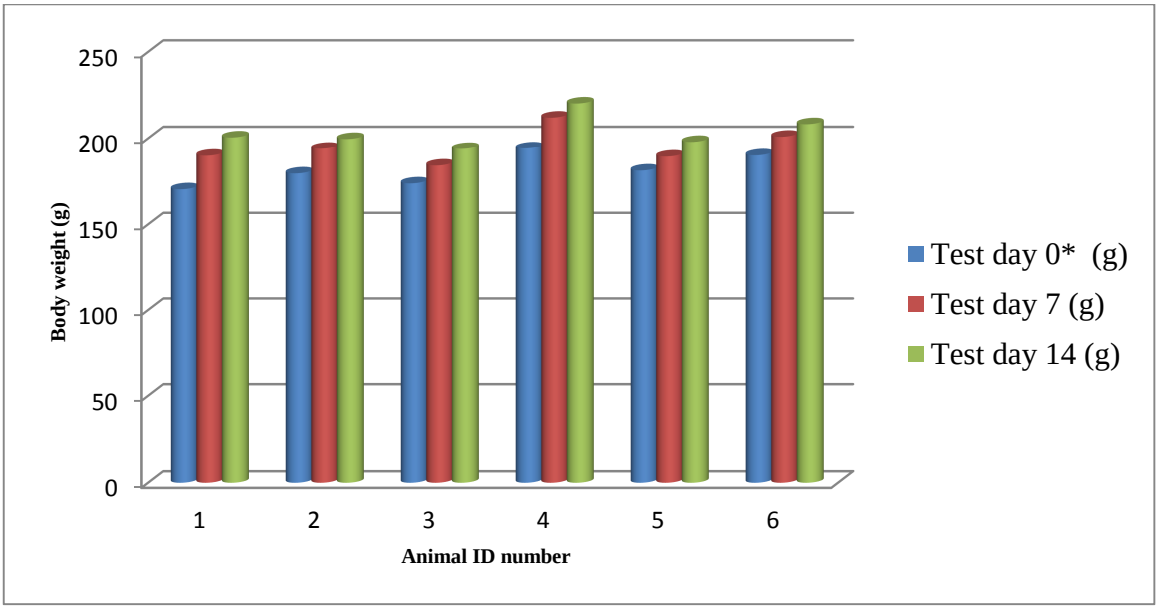
No mortality was observed in animals of both the groups. All the animals had gained body weight by day 14 as compared to day 0 (**Table 2, Fig.1**). The average weight gains observed in the animals are considered to be within the range of commonly recorded body weight in this strain.

In Group I, hydrometra was observed in one animal. No abnormalities were observed for rest of the Group I and Group II animals at terminal sacrifice (**Table 3**).

From the results it was observed that after administration of the extract animals appeared dull for brief time duration (30 min and 1 h in Group I and 30 min in Group II) after which they became normal throughout the experimental procedure (till 14 days). This temporary dullness indicates a possible role of the extract in neuro-suppression. Thus it can be used as a drug in treatment of neurological disorders characterized by the hyper activity of neurons.

Hydrometra was observed in one rat during the experiment. This is common in female rats due to luminal dilatation of uterus most commonly due to accumulation of secretions from normal cyclical changes of uterus. Interestingly, no mortality was observed in the animals of both the groups. Moreover, all the animals had gained body weight during the experimental procedure. This establishes that the L-DOPA enriched extract of *Mucuna. pruriens* is non cytotoxic to the rats.Based on the results, the median lethal dose (LD_{50}) of extract after single oral administration to female rats, observed over a period of 14 days is greater than 2000 mg/Kg body weight.

Figure 1 Graphical representation of changes in body weight of experimental rats with *Mucuna pruriens* seed extract (2000 mg/Kg body weight) at 0, 7, 14 days



CONCLUSION

Mucuna pruriens seed extract was assessed for its oral toxicity in the rats. It was found that the high dosage (2000mg/Kg body weight) of the extract was non toxic to rats and helped in weight gain. Additionally, a brief period of dullness indicates a possible role of the extract in neuro-suppression as well. Thus it can be used as a drug in treatment of neurological disorders characterized by the hyper activity of neurons. To summarize, the results of this study collectively specify that oral administration of *Mucuna pruriens* is not connected with any toxicologically 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.

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