



## EVALUATION OF ANTIPARKINSON'S ACTIVITY OF INDONESIAN VELVET BEAN (*Mucuna pruriens*) EXTRACT

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### ABSTRACT

The purpose of this research was to conduct pharmacological studies of antiparkinson's activity of Indonesian *Mucuna pruriens* extract in mice. Effect of ethanolic extract of *Mucuna pruriens* was studied using in-vivo parameters, i.e. catalepsy and transfer latency. The ethanolic extracts of *Mucuna pruriens* were assessed at two dose levels (200 and 400 mg/kg) in haloperidol-induced mice. The results showed that *Mucuna pruriens* extract decreases the cataleptic symptoms and transfer latency score. The *Mucuna pruriens* extract at a dose level of 200 mg/kg was better than those at a dose level 400 mg/kg in reduce catalepsy and transfer latency scores. *Mucuna pruriens* extract were considered to be safe up to a dose level of 5000 mg/kg based on the acute toxicology test.

**Keywords:** antiparkinson, mucuna pruriens, haloperidol, catalepsy, transfer latency.

### INTRODUCTION

Parkinson's disease is a neurodegenerative disease that occurs in dopaminergic neurons due to reduction of dopamin level in the brain. After Alzheimers Disease, Parkinson's disease is the one of the most popular neurodegenerative disease (Kabra *et al.*, 2014). On average, one in every 250 people above 40 was diagnosed with this disease (Sivaraman *et al.*, 2010). Parkinson disease indicates four main motoric symptoms, i.e. tremor, rigidity, bradykinesia, and postural instability. In addition, Parkinson disease also shows non-motoric symptoms such as sensoric disturbances, neurobehavioral disturbances (depression, anxiety, and psychosis), and decreased ability to remember (Joshi *et al.*, 2011). Recently, pharmacological treatment for Parkinson disease has use synthetic L-Dopa. However, long-term treatment using synthetic L-Dopa is often generated negative side effects such as euphoria, halutinations, and phobias (Mena *et al.*, 1997)

Herbal medicine is now getting more attention because of its potential of myriad benefits to the community, especially in medicine (Sandhya *et al.*, 2010). The World Health Organization (WHO) is taking an great interest in herbal medicines in order to facilitate its aim of making health available for all. Herbal medicine has remained popular in throughtout the world, primary for the developing countries because of its affordability, accessibility, and advantage of having several efficacy and minimal side effects (World Health Organization, 2013). In line with the WHO policy, herbal medine for Parkinson disease is very important to be developed.

*Mucuna pruriens* is a medicinal plant from the tropics. In Indonesia, this plant is found in Central Java. It belongs to the family *Fabaceae* and can grow up to 18 meters in height. *Mucuna pruriens* is also popular as a medicinal plant in India. The root, leaf, and seeds of *Mucuna pruriens* are typically used to treat impotence, snake bite, diabetes, and tremors in India. An extract of

*Mucuna pruriens* seeds was used to effectively treat tardive dyskinesia which is a kind of neurodegenerative disease (Sivaraman *et al.*, 2010).

Previous researches have characterized the components of *Mucuna pruriens*. Misra and Wagner (2007) showed that L-Dopa from India's *Mucuna pruriens* had up about 7-10% of unripe seeds and 3-6% of ripe seeds. Other biochemical components of *Mucuna pruriens* have been reported: protein (20-29%), lipids (6-7%), fiber (8-10%), and carbohydrate (50-60%) (Mohan and Kala, 2010). The leaf of *Mucuna pruriens* was reported to contain L-Dopa (~0.5%) (Desai *et al.*, 2010). The cotyledon seeds of *Mucuna pruriens* from Indonesia have been shown to contain L-Dopa 7.56% of L-Dopa, whereas shells contain only 3.89% (Sardjono, 2012). Because it contains L-Dopa, the seed of *Mucuna pruriens*, particularly from Indonesia, has the potential to be used as an herbal medicine for Parkinson's disease. The present study was carried out to evaluate the anti-Parkinson's activity of ethanolic extract of seeds of Indonesian *Mucuna pruriens* on mice that induced by haloperidol.

### EXPERIMENTAL SECTION

#### Materials

The seeds of *Mucuna pruriens* were collected from Central Java, Indonesia. The chemical used included: ethanol (98%), citric acid, aquabidest, phosphoric acid (H<sub>3</sub>PO<sub>4</sub>) p.a, methanol p.a, standard L-Dopa (3, 4-dihydroxy-L-phenylalanine), and PGA (Pulvis Gummi Arabicum) 1%.

#### Instrumentation

High Performance Liquid Chromatography (HPLC) Shimadzu used to determine L-Dopa content of *Mucuna pruriens*. Analysis of L-Dopa was carried out at 280 nm by using a chromosil C18 reverse phase column of 250 x 4.6 mm. The mobile phase consisted of water,



methanol and phosphoric acid in the ratio of 97 ml : 20 ml : 1 ml or 82.20:16.95:0.85 and flow rate of 1 mL/min.

## Procedure

### Plant extraction

Prior to using, freshly collected *Mucuna pruriens* seeds were separated from the peel and dried in sunlight. Thus, freshly prepared *Mucuna pruriens* seeds were pulverized. *Mucuna pruriens* seeds powder (15 Kg) was subjected to a maceration extraction process in a sufficient water:ethanol (1:1) solvent (up to pH 3) for 3 days. During the extraction process, the solvent was changed every 24 hours and the extract were separated from the solvent using a rotary vacuum evaporator. A brownish-black aqueous residue was obtained and dried using a freeze dryer. The percentage yield of the ethanolic extract was 1.58%.

### L-Dopa content measurement

The amounts of L-Dopa in the extracts were determined using High Performance Liquid Chromatography (HPLC) (Upadhyay, 2012). The amount of L-Dopa in the extract was determined using mobile phase combination of water, methanol, and phosphoric acid. Standard L-Dopa (12.5 g) was dissolved in 25 mL of the mobile phase to obtain a primary standard solution of 500 ppm. A standard solution series of concentrations of 25, 50, 75, 100, 125 and 150 ppm was prepared. For the sample solution, 12.5 mg of the *Mucuna pruriens* extract was dissolved in 10 mL of the mobile phase, homogenated using ultrasonic vibrator, and diluted to 25 mL in a volumetric flask.

### Animals

Mice (*Mus musculus*) of either sex weighing 20-30 g were used for the study. The animals were maintained in a ventilated room with a 12-hour light/dark cycle in standard polypropylene cages under room temperature ( $25 \pm 1^\circ\text{C}$ ). They were fed a standard pellet diet throughout the experimental period. The animals underwent adaptation to the polypropylene cages for a week prior to the experimentation period.

### Acute toxicity test

Twenty male and female mice weighing 20-30 g were used in the acute toxicity test. The animals were only provided water for the 12 hours prior to testing, after which the *Mucuna pruriens* extract was administered orally at a dose of 1000 mg/kg of body weight. If mortality was not observed, the procedure was repeated with a higher dose of 2000 mg/kg; 3000 mg/kg; 4000 mg/kg; and 5000 mg/kg of body weight. The dose level resulting in mortality was recorded as the toxic dose.

### Behavioral assesment of catalepsy

Twelve albino mice of either sex (weighing 20-30 g) was divided into five groups of three animals each ( $n=3$ ). Group 1 received a PGA 1% solution and served as control (vehicle). Groups 2, 3, 4 and 5 received

haloperidol (5 mg/kg) with, or without extract 30 minute after intraperitoneal administration of the vehicle. Group 2 served as negative control receiving no extract. Group 3 received L-Dopa as positive control, Groups 4 and 5 received extract at a dosage of 200 and 400 mg/kg respectively. The cataleptic levels of the animals were observed by a modification of the Costall and Olley method (Subarnas *et al.*, 1993). Catalepsy score was measured for each 30 minutes up to 2 hours after haloperidol administration, by gently placing both the forepaws of the mouse over a metal bar (diameter 0.5 cm) suspended 15 cm above the table top (Figure-1).

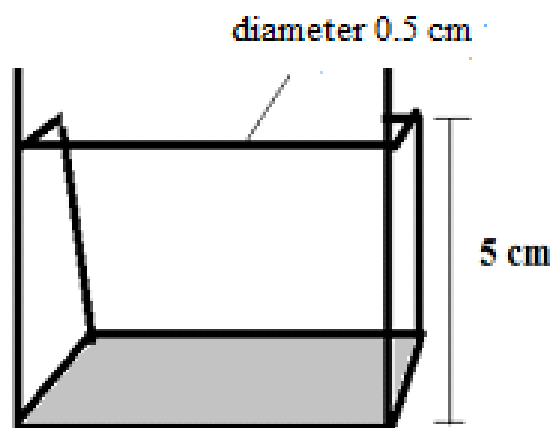


Figure-1. Scheme of catalepsy test.

The intensity of catalepsy was assessed by counting time in seconds until the mouse brought both forepaws down to the table top, with a by counting time in seconds until the mouse brought both forepaws down to the table on the high bar. The catalepsy test was carried out 30 minutes after administration of haloperidol. Mice were categorized to be cataleptic if they failed to move more within 15 seconds.

### Transfer latency test

The same animals were tested for transfer latency of 24 hours after the administration of haloperidol and *Mucuna pruriens* seeds extract. Transfer latency tests were carried out in a labyrinth using a modification to the Marklund method (Marklund and Marklund, 1974) shown in Figure-2. Transfer latency is a long-term spatial memory measurement based on the time it takes for the mice to move from the start to the finish area at the end of a labyrinth. The labyrinth size used was 100 cm x 50 cm. If the mice failed to move towards the end of the labyrinth within 90 seconds, they were scored the maximum transfer latency time (90 seconds).

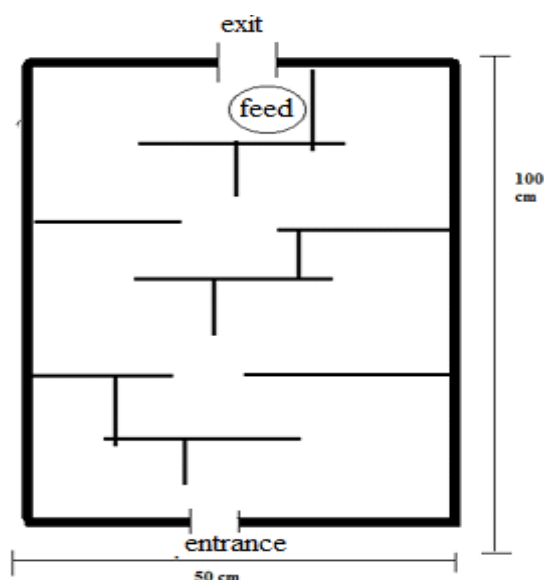


Figure-2. Scheme of labyrinth.

### Statistical analysis

The Statistical analysis was performed using One Way ANOVA followed by Dunnet's comparison test and student t-test (unpaired). The values were expressed as mean and the  $P < 0.05$  was taken as significant.

## RESULTS AND DISCUSSIONS

### L-Dopa content measurements

HPLC data of standard L-Dopa chromatogram showed in Table-1. Based on the standard L-Dopa chromatogram, L-Dopa peaks were showed at retention time 3.44 minutes (sample no.1-5). That data was used as a hint for L-Dopa determination in the extract. *Mucuna pruriens* extract sample chromatogram (sample no.6) showed that there were several peaks rise in retention time from 1.37 to 3.41 minutes. The peak at the retention time of 3.41 from *mucuna pruriens* extract chromatogram is within the range of the standard L-Dopa. Therefore, *Mucuna pruriens* extracts expected to contain L-Dopa and its content were calculated by calibration curve between standard series and sample. From the calculation, content of L-Dopa in the sample of *Mucuna Pruriens* extracts was about 13.9 %.

Table-1. The Results of HPLC measurement.

No	Sample	Retention time	Peak area	Concentration (%)
1	Standar (L-Dopa) solution 25 ppm	3.44	271752	100
2	Standar (L-Dopa) solution 50 ppm	3.44	531839	100
3	Standar (L-Dopa) solution 75 ppm	3.44	1091078	100
4	Standar (L-Dopa) solution 100 ppm	3.44	1365386	100
5	Standar (L-Dopa) solution 125 ppm	3.44	1679937	100
6	<i>Mucuna pruriens</i> extract solution (0.0133g/25 mL)	1.37	33786	2.275
		1.62	29026	1.954
		1.78	34027	2.291
		2.31	42235	2.843
		2.70	11521	0.776
		3.21	412849	27.795
		3.41	921905	62.066

### Acute toxicity test results

Administration of *Mucuna pruriens* seeds extracts did not lead to mortality in the mice (male and female) at a dose level of 1000 mg/kg body weight up to at a dose level 5000 mg/kg body weight (Table-2). Thus, a 5000 mg/kg body weight dose of extracts can be considered safe. In the pharmacological study (catalepsy and transfer latency test, dose levels of 200 and 400 mg/kg were selected, as they were well within a safe range (non toxic).

Table-2. Acute toxicity test results.

No	Dose levels*	Amount of mice		Mortality	
		Male	Female	Male	Female
1	1000	10	10	0	0
2	2000	10	10	0	0
3	3000	10	10	0	0
4	4000	10	10	0	0
5	5000	10	10	0	0

\*mg/kg of body weight



### Behaviour evaluation of antiparkinson

#### Anticataleptic activity of *Mucuna pruriens* extract

In order to evaluate its pathophysiological mechanisms and for exploring potential treatments, an animal model was used in Parkinson pharmacology test activity (Dawson, 2000; Rodriquez *et al.*, 2001). Typically, models of Parkinson disease were characterized by akinesia observation, such as in bar test for rigidity or catalepsy. Neuroleptics, such as haloperidol, can produce a sustained but reversible akinesia, due to blockade of

dopamine receptors. This neuroleptic-induced Parkinsonism is a major side effect of their use in treatment of schizophrenia.

Neuroleptics have been utilized as an acute model of Parkinson (Kabra *et al.*, 2014). The central dopaminergic function and evaluation of dopamine agonistic activity was carried out by observing the cataleptic behavior in mice. Haloperidol blockades the dopamine receptors in the brain and produces the extra pyramidal side effect.

**Table-3.** Catalepsy test results.

Treatment	Catalepsy score ( seconds)				
	0	30'	60'	90'	120'
Vehicle (normal)	1.95±0.76	2.25±0.56	1.95±0.76	3.83±1.45	2.67±0.82
Negative Control (Haloperidol)	2.25±0.56	21.42±3.45	22.58±6.61	27.25±7.63	24.75±5.07
Positive Control (L-Dopa)	1.97±0.56	2.22±0.51	2.33±0.25	2.55±0.44	2.46±0.18
Dose 1 (200 mg/kg)	2.67±0.82	3.99±1.72	4.57±2.03	3.46±0.95	3.67±1.53
Dose 2 (400 mg/kg)	2.25±0.56	6.59±1.95	7.62±1.79	6.38±2.16	5.25±2.28

The results of an anticataleptic studies are shown in Table-3. Negative control group was haloperidol-induced mice. Haloperidol can trigger cataleptic symptoms (Abdel-Salam, 2012). Treatment with L-Dopa showed a significant ( $P<0.01$ ) reduction in the cataleptic behavior between 30 to 120 minutes of time interval as compared to the haloperidol treated group. Treatment with *Mucuna pruriens* extract showed a significant ( $P<0.01$ ) reduction in the duration of cataleptic behavior dose dependently when compared to haloperidol treated group.

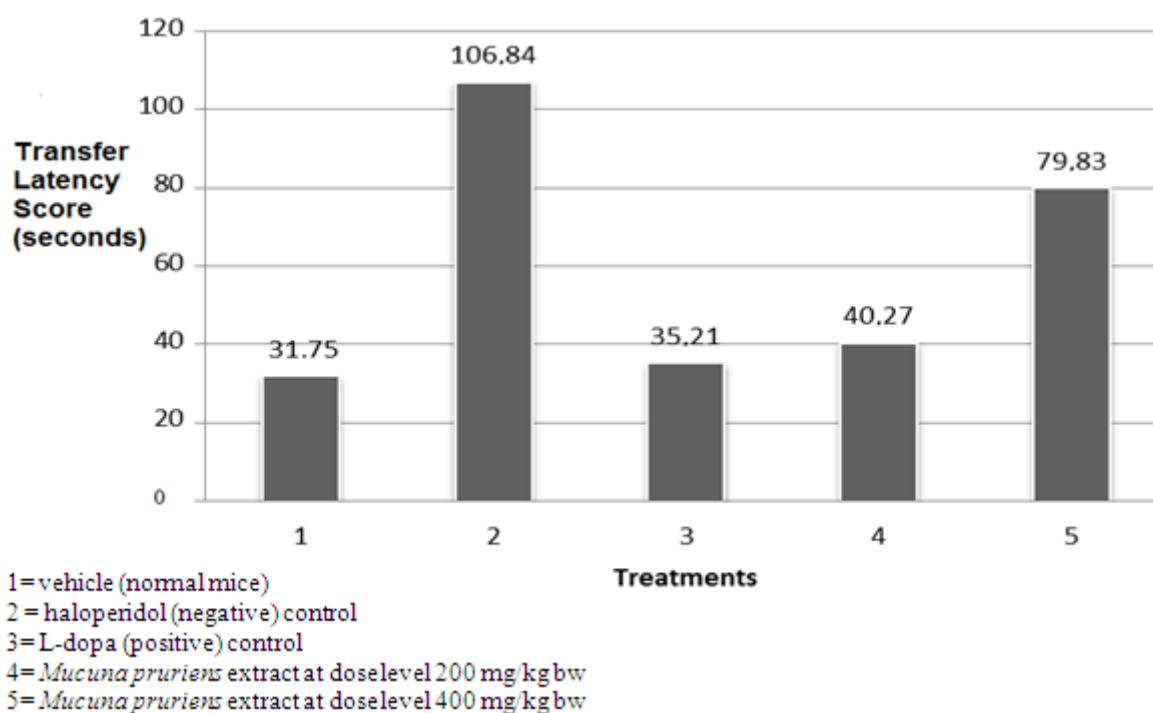
Table-3 shows that the animal's cataleptic symptoms occur by 30 minutes in continue through 120 minutes of the test. *Mucuna pruriens* extract at a dose 200 mg/kg of body weight reduced cataleptic symptoms which was almost the same as the normal condition (vehicle) and positive control (L-dopa treatment). *Mucuna Pruriens* seeds extract at a dose level of 400 mg/kg of body weight also could reduced the cataleptic score significantly ( $P<0.05$ ) in compare to negative control. The statistical comparison between *Mucuna pruriens* extract dose level indicated that 200 mg/kg dose has smaller P value (0.001) than 400 mg/kg (0.002). The 200 mg/kg dose of *Mucuna*

*pruriens* extract has more significant effect in reduced cataleptic symptoms than 400 mg/kg

#### Transfer latency test results

The same experimental groups of mice tested for transfer latency test. The average transfer latency time data for treatment with *Mucuna pruriens* extracts at 200 and 400 mg/kg of body weight showed in the Figure-3. Similarly to the catalepsy test, haloperidol was used as a negative control and L-Dopa treatment used as a positive control. Transfer latency is scored in seconds. If the mice have a lower transfer latency score, it means that the mice have a good ability to remember a labyrinth route. Figure-3 shows that treatment using *Mucuna pruriens* extract at a dose of 200 mg/kg has a lower transfer latency score in comparison to 400 mg/kg body weight

The extract at a dose level 200 mg/kg of body weight increased the mice ability to remember. Similarly to experiment at a dose level 200 mg/kg of body weight, treatment group at a dose level 400 mg/kg of body weight also could reduced transfer latency scores in comparison to negative control.



**Figure-3.** The Results of transfer latency test.

Statistical analysis indicated *Mucuna pruriens* extract at a dose level 200 mg/kg have a P value 0.018 ( $P < 0.05$ ) in comparison to negative control. That means that this dose of *Mucuna pruriens* extract showed significant decrease the transfer latency score when compared to the haloperidol (negative) group, but the effect of this dose on the L-dopa treated group (positive control) was found to be insignificant.

*Mucuna pruriens* extract at a dose level 400 mg/kg have a P value 0.945 ( $P > 0.05$ ) in comparison to negative control which means that it was insignificant from negative control. So that, the extract at a dose level of 200 mg/kg of body weight have better influence than the extract at a dose level of 400 mg/kg.

## CONCLUSIONS

HPLC analysis of the *Mucuna pruriens* seed extract from Central Java Indonesia has shown to have L-Dopa content of about 13.9%. Our results showed Indonesian *Mucuna pruriens* extract reduced cataleptic symptoms and transfer latency score. The Indonesian *Mucuna pruriens* extract can be considered to be a potential herbal source for treating Parkinson disease. In the future work, the researcher should manipulate more various doses of *Mucuna pruriens* extract and find the optimum dose.

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