ANTI-TUSSIVE EFFECT AND GROSS TOXICITIES OF METHANOL EXTRACT OF *MUCUNA PRURIENS* (L) DC IN COMPARISON OF CODEINE PHOSPHATE

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ABSTRACT

The aim of the current study is to evaluation of plant seed extract of *Mucuna pruriens* for its gross toxicities and antitussive activity. Anti-tussive effect was observed by inducing cough using sulfur dioxide gas and counts the number of cough before and after administration of extract and centrally acting cough suppressant (codeine phosphate) given orally. *Mucuna pruriens* seed extract showed highly significant antitussive effect (**p<0.005) and compare to control and reference drug codeine phosphate and may act on opioid receptor that may located in airway passage and produces inhibitory effect. The results suggest that *Mucuna pruriens* seed may contains cough protective agent and have potential therapeutic value.

Keywords: Anti-tussive effect, gross toxicities, codeine phosphate, mice, *Mucuna pruriens*.

INTRODUCTION

Treatment with the herbal medicines is not new they are used from thousands of years¹. Total flowering plants are approx. 4, 22,000 from which 50,000 have medicinal values².

In under developed countries traditional herbal drugs have a great demand due to their lesser adverse effects, efficacy and low cost³ and for biological and pharmacological assessment at least 20% of plants are used⁴ and they play an important role in the cure of respiratory diseases but clinical trials are required to assess the role of medicinal herbs. In developing countries 80% of people depend on natural products especially from plants for their health issues⁵.

Herbal medicines have major role for prevention and treatment of diseases⁶ in various ancient traditional system of medication, despite advances in modern pharmaceutical medicines, herbal drugs still play an important in preventive and curative of diseases. Commercially medicinal plants are cultivated for extraction of some important active constituents. Furthermore, active constituents give better result when it combines with other constituents⁷.

Mucuna pruriens (L) DC family *Fabaceae*, is a climbing legume⁸ and well-known plant in ancient practices⁹ commonly known as Kapikachu or Velvet bean widely distributed in Pakistan, India, Sri Lanka, Burma, Africa¹⁰. The plant is 15m in length, climbing and annual shrub. Seeds are shiny black or brown. Young plant is completely covered with hairs and when older, hairs are completely removed. Seedpods are covered with loose orange hairs that are responsible for severe itch⁹. Hair of the *Mucuna pruriens* contains 5-hydroxytryptamine, protein and mucunain¹¹ that causes severe itching. Other constituents of *Mucuna pruriens* are L-DOPA¹⁰ crude protein, 20-29 %, niacin, nicotine, total dietary fibre 8-10%, crude fat, ash 3%, carbohydrates 50-60%, valine, cystine, methionine, threonine, tryptophan, tryptamine, tyrosine, vernolic acid, water, leucine, oleic acid, linoleic acid, palmitic acid, trypsin, saponins, riboflavin and flavonoids.

Plant used in the treatment of Parkinson's disease¹² because seeds contain high concentration of levodopa i.e a direct precursor of the neurotransmitter dopamine¹³. The hairs of the plant are used as vermifuge (Krishnan, 2000). In infertile

men Plant seeds are helpful to increase the sperm concentration and motility¹⁴. It has anti-anaemic⁸, anti-fungal¹⁵, anthelmintic¹¹, anti-protozoal¹⁶, anti-microbial¹¹, anti-diabetic¹⁷, neuroprotective¹³, antioxidant¹⁸, hypocholesterolemic¹⁹, aphrodisiac²⁰ and hypoglycemia properties²¹. In addition, it has a prophylactic activity in snakebite²² and helpful against cobra venom-induced cardiotoxicity²³ and, cough and cold. Body takes to get rid of substances that are responsible to irritating the air passages is a cough. In acute cough that may be 15- 20 days can be caused by an infection and noninfectious include pneumonia, viral upper respiratory infections, whooping cough and acute bronchitis²⁴ and chronic cough that is more than 20 days²⁵. Chronic cough may be due to conditions within the lungs, environmental irritants, chest cavity conditions or condition of the passages that transmit air.

MATERIALS AND MATHODS

Extract Preparation

The seeds of *Mucuna pruriens* were collected from the month of May and soaked in methanol for ten days. The methanol was filtered and the extract was concentrated by means of evaporation, thick gummy mass was collected. The concentrated mass of *Mucuna pruriens* seeds was used for antitussive activity and gross toxicities.

Animals Selection

Albino mice (25-30 gm) of both sex were used and maintained at 22 ± 1 °C with light/ dark cycle of twelve/ twelve hours or from 08.00 a.m to 08.00 p.m at the Department of Pharmacology, University of Karachi. They were housed two per cage in under standard environmental conditions and kept at least 4 – 5 days before start of experiment.

Dosing

The animals were divided into five groups (n=7). One group served as control group received saline 0.9%, three groups of seed extract of *Mucuna pruriens* in the doses of 300, 500 and 1000 mg/kg and last group received standard drug codeine phosphate (10 mg/kg).

Assessment of Gross Toxicity

The gross toxicities were assessed on weekly basis for 30 days and noticing awareness, alertness, stereotype, mood, vocalization, aggression, tremors, spontaneous activity, fearfulness, corneal reflex, light reflex, touch response, body movement, pain response, sedation, grip strength, body tone, limb tone, passivity, irritability, piloerection, staggering gait, startle response and note the number of animals died during the total period of experiment.

Antitussive activity

Antitussive activity of plant extract was determined by using a sulfur dioxide gas-induced cough in mice²⁶. During experiment concentrated sulfuric acid and aqueous saturated sodium hydrogen sulfite solution were introduced in apparatus to produce the sulfur dioxide gas and pressure of gas recorded by mercury manometer.

Initially, at 0 minute the cough response of all the groups was observed by placing the animals in sulfur dioxide gas-induced cough apparatus one by one. Animal was taken out from the apparatus after one minute of introducing the gas. Frequency of cough was observed for five minutes. In the same way the frequency of cough before drug administration were observed for all the animal groups at 0 minutes and after drug administration at 30, 60, 90 and 120 minutes intervals.

Statistical Analysis

All results were expressed as average value $\pm$ standard deviation (St.Dev). The significance of difference between averages was determined²⁷⁻²⁸. The data obtained from present study was analyzed for P-value < 0.05 was considered as significant and P-value < 0.005 was considered as highly significant, following the one way ANOVA.

Table 1: Gross Behavioral Activities

Activities	Increase (Days)			Decrease (Days)			No Change (Days)			Present/Absent (Days)		
	07	15	30	07	15	30	07	15	30	07	15	30
Awareness	-	-	-	*↓	*↓	*↓	-	-	-	-	-	-
Alertness	-	-	-	*↓	*↓	*↓	-	-	-	-	-	-
Stereotype	-	-	-	-	-	-	NIL	NIL	NIL	-	-	-
Mood	-	-	-	*↓	*↓	*↓	-	-	-	-	-	-
Vocalization,	-	-	-	-	-	-	NIL	NIL	NIL	-	-	-
Aggression	-	-	-	-	-	-	NIL	NIL	NIL	-	-	-
Tremors	-	-	-	-	-	-	NIL	NIL	NIL	-	-	-
Spontaneous activity	-	-	-	*↓	*↓	*↓	-	-	-	-	-	-
Fearfulness	-	-	-	-	-	-	-	-	-	-ve	-ve	-ve
Corneal reflex	-	-	-	-	-	-	NIL	NIL	NIL	-	-	-
Light reflex	-	-	-	-	-	-	NIL	NIL	NIL	-	-	-
Touch response	-	-	-	*↓	*↓	*↓	-	-	-	-	-	-
Body Movement	-	-	-	*↓	*↓	*↓	-	-	-	-	-	-
Pain response	-	-	-	*↓	*↓	*↓	-	-	-	-	-	-
Sedation	-	-	-	-	-	-	-	-	-	+ve	+ve	+ve
Grip Strength	-	-	-	*↓	*↓	*↓	-	-	-	-	-	-
Body tone	-	-	-	*↓	*↓	*↓	-	-	-	-	-	-
Limb tone	-	-	-	*↓	*↓	*↓	-	-	-	-	-	-
Passivity	-	-	-	-	-	-	-	-	-	+ve	+ve	+ve
Irritability	-	-	-	-	-	-	-	-	-	-ve	-ve	-ve
Piloerection	-	-	-	-	-	-	-	-	-	-ve	-ve	-ve
Staggering Gait	-	-	-	-	-	-	-	-	-	-ve	-ve	-ve
Startle response	-	-	-	-	-	-	-	-	-	-ve	-ve	-ve

*↓p<0.05 indicates significant reduction; +ve indicates Present; -ve indicates Absent

Figure 1: Effect of *Mucuna pruriens* at the dose of 300 mg/kg

n = 7, Average value $\pm$ St. Dev; Significant difference by Newman Keuls test; **p < 0.005 as compared to control rabbits, following one way ANOVA

Figure 2: Effect of *Mucuna pruriens* at the dose of 500 mg/kg

n = 7, Average value $\pm$ St. Dev; Significant difference by Newman Keuls test; **p < 0.005 as compared to control rabbits, following one way ANOVA

Figure 3: Effect of *Mucuna pruriens* at the dose of 1000 mg/kg

n = 7, Average value $\pm$ St. Dev; Significant difference by Newman Keuls test; *p < 0.05 as compared to control; **p < 0.005 as compared to control rabbits, following one way ANOVA

RESULTS

Assessment of Gross Toxicities

No gross toxicity was observed in any group of animals during the total period of experiment. Animals showed significant (*p<0.05) reduction in pain response, grip strength, body tone, limb tone, awareness and alertness with significant (*p<0.05) increase in sedation during the total period of experiment and showed significant (*p<0.05) increase in passivity with no significant change in righting reflex after administration of *Mucuna pruriens* (Table 1) and no death occur during the total period of experiment.

Antitussive activity

Mucuna pruriens extract (300, 500 and 1000 mg/kg) showed significant cough suppressive activity. No cough suppressive effect was seen at the dose of 300 mg/kg with percentage inhibition of 75.0 % (at 30 min), 70.60 % (at 60 min), 69.0% (at 90 min), and 68.30 % (at 120 min) in Figure 1.

It showed marked cough suppressive activity at 500 mg/kg with percentage inhibition 37.9 % (at 30 min), 30.7 % (at 60 min), 14.9% (at 90 min), and 7.7 % (at 120 min) in Figure 2. Results were obtained at 1000 mg/kg with percentage inhibition 39.90% (at 30 min), 31.70% (at 60 min), 15% (at 90 min), and 18.70 % (at 120 min) in Figure 3.

Codeine phosphate produced 38.7 % (at 30 min), 30.7 (at 60 min), 26.4 % (at 90 min) and 17.5 % (at 120 min) inhibition of cough frequency in animals. The comparison of the antitussive effect of Codeine phosphate and time interval is shown in Figures 1-3.

DISCUSSION

Herbal drugs have potential source of therapeutics and help in health care system for human beings all over the world. And further research of medicinal herbs is necessary to isolate the active compounds responsible for the biological activities⁵. *Mucuna pruriens* was selected for present study because of its medicinal value and some previous research studies such as seeds are used as alternative to L-DOPA and relieve pain and fever²⁹.

In gross toxicities, animals showed no significant change in corneal reflex, light reflex, tremor and animals became passive after administration of *Mucuna pruriens* at the dose of 500 mg/kg. It was also noted that the plant seed extract is affected on pain and touch responses. It significantly reduces (*p<0.05) the touch response and body movement with significant reduction (*p<0.05) in awareness, alertness, and locomotion. It also significantly reduces (*p<0.05) the grip strength and body tone, it could be due to the deficit motor

coordination and muscle tone occurred during administration of *Mucuna pruriens*.

In gross toxicity tests plant seed extract showed sedation and decrease pain and grip strength may indicated its cough suppressive effect and extract of *Mucuna pruriens* seeds has been reported to be useful in cough and cold and are used as antitussive agent³⁰.

The frequency of cough of control group ranges between 74.0±0.012 and 69.50 ± 0.016 (mean ±S.E.M) and the frequency of cough of extract group ranges between 39.90±0.012 and 7.70 ± 0.026 (mean ±S.E.M). The antitussive effect was highly significant (**p<0.005) at 500 and 1000 mg/kg, and non-significant at the dose of 300 mg/kg.

In the present study the extract of *Mucuna pruriens* after oral administration decrease the cough frequency similar to that of codeine phosphate. The effect of *Mucuna pruriens* administered at the highest doses caused a significant inhibition of cough reflex comparable to codeine phosphate (10 mg/kg). It has efficacious value in experimental cough and is widely available over-the-counter as a cough suppressant.

In another study asterosaponin has an expectorant, antitussive and anti-asthmatic effects and decreased the frequency of cough caused by ammonia in mice and asthma induced by histamine³¹ and *Mucuna pruriens* contains flavonoids and saponin that are involve for the therapeutics of respiratory diseases and chronic bronchitis. So antitussive effect of herbal drug could be due to the effect of *Mucuna pruriens*'s constituent flavonoid and saponin.

Mucuna pruriens may act directly on opioids receptors as codeine. Opioids receptors have multiple subtypes³² and codeine binds with mu (μ) receptor within CNS and alters the processes of emotional pain response by affecting the perception of pain. And *Mucuna pruriens* has an analgesic effect also. So *Mucuna pruriens* could be act on opioids or mu (μ) receptor as codeine that may locate in tracheobronchial passage, produces inhibitory action on opioids.

It is concluded that the *Mucuna pruriens* is non-toxic and has a potential of centrally acting cough suppression and can be use safely as an anti-tussive agent.

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