

CNS Activity of the Methanol Extracts of *Solanum Pubescens* in Experimental Animal Model

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Abstract: The aim of this present study is to investigate central nervous system activity of the methanol extract of leaves of *Solanum pubescens* in Swiss Albino mice and Wistar albino rats. *Solanum pubescens* is a traditional medicine plant for the treatment of Hypoglycemic, Anti-bacterial & Anti-HIV activities. The work reached the acute toxicity studies *solanum pubescens* and its action on the central nervous system, because no data in the literature have been found of pharmacological activity of this plant in the central nervous system. The leaves was extracted with methanol and investigated for its central nervous system activity of Albino mice in Rota-rod and Actophotometer at the dose level of 300mg/kg. The extract exhibited significant central nervous system activity. This study established central nervous system activity in *Solanum pubescens* leaves. It has been concluded that methanolic extract of *Solanum pubescens* exhibited Anxiolytic, Myorelaxant & Anti-depressant activity.

Keywords: *Solanum pubescens*, Anti-anxiety, Anti-depressants, Myorelaxant.

I. Introduction

Solanum pubescens

Solanum pubescens belong to the family solanaceae commonly called as pajarito and commonly used in India by the tribal people for the treatment of liver disorders¹, diarrhoeal diseases and cancer disorders. However, there are no reports on the central nervous system activity of this plant, although decoction was extensively used by the tribes in hilly region, to reduce mental tension and also induce sleep. Therefore in the light of their reported use in traditional medicine as Anti-anxiety, Anti-depressant and myorelaxant agent, the present study was under taken for the first time to investigate central nervous system activity of the menthol extract of *solanum pubescens* in experimental animal models.

II. Material and Models

Plant materials and extraction:

The leaves of *Solanum pubescens* (solanaceae) was collected in march 2010 from S.V.university, Tirupathi, Andhra pradesh, India. The plant material was taxonomically identified by the botanist Dr. K. Madhavachetty and the voucher specimen was retained in our laboratory for future reference. The dried powder material (500g) of the leaves of *Solanum pubescens* was extracted with 2000ml of methanol in a Soxhlet apparatus. The methanol extract was distilled, evaporated and dried in vaccum. The resulted extract yield was 7.45% and the appearance of the extract was dried gum resin in nature. The chemical constituents of the extract were identified by qualitative analysis followed by their conformation through the literature.

Experimental Animals:

Studies were carried out using Swiss Albino mice (20-25g) and Wister Albino rats (150-180g) of either sex. They were obtained from the animal house, NIN, Hyderabad, India. The animals were grouped and housed in polyacrylic cages (38×23×10cm) with not more than eight animals per cage and maintained under standard laboratory conditions temperature (25± 2°C) with dark and light cycle (14/10hr). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water ad libitum. The mice were acclimatized to laboratory condition for 10 days before commencement of experiment. All procedures described were reviewed and approved by the animal's ethical committee.

Preliminary phytochemical analysis:

The Methaol extract of *solanum pubescens* was subjected to preliminary phytochemical screening².

Drugs:

Diazepam (lupin laboratories Ltd. India), is used as a standard drug for all the models.

Dose selection:

The dose was selected according to the data obtained from the literature.

Exploratory behavior:

This was performed in by (i) Y-maze test
(ii) Head dip test

Y-maze test:

This was performed in the groups of 6 Albino mice at 30, 60, 90 and 120 min after injection of either propylene glycon (5ml/kg), menthol extract of *solanum pubescens* 300mg/kg of diazepam (5mg/kg) respectively. The mice were placed individually in a symmetrically Y-shaped runway (33cm×38cm×13cm) for 3min and the number of the maze with all four feet (an entry) were counted³.

Head dip test:

Three groups of Albino mice (n=6) were placed on top of a wooden box with 16 evenly placed holes, 30 min after injection of the methanol extract of *solanum pubescens* 300mg/kg, vehicle 5ml/kg and diazepam 5mg/kg respectively. The number of times that each animal dipped its head into the hole was counted for the period of 3min⁴.

Muscle relaxant activity:

The effect of extracts on muscle relaxant activity was studied by the

- a) Traction test
- b) Rota-rod test

Traction test:

Placing the fore paws of the mice in a small twisted wire rigidly supported above the bench top for the screening of the animal. Normally the mice grasp the wire with the forepaws, and place at least one hind foot on the wire without 5 second when allowed to hang free. The test was conducted on 3 groups of animals (n=6) that were previously screened, 30 min after the injection of methanol extract of *solanum pubescens* (300mg/kg), diazepam (5mg/kg), or control (5ml/kg) as a vehicle. Inability to put up at least one hind foot considered failure in the traction test⁵.

Rota-rod test:

Fresh mice were placed on a horizontal wooden rod (32mm diameter) rotating at a speed of 5rpm. The mice capable of remaining on to top for 3min or more, in three successive trials were selected for the study. The selected animals were divided into 3 groups (n=6). Methanolic extract of *solanum pubescens* at the dose of 300mg/kg were injected intra peritoneally into group I, group II, received control 5ml/kg and group III, received diazepam 5mg/kg was administered. Each group of animals was then placed on the rod at an interval of 30, 60, 90, 120 and 150 min. The animals failed more than once to remain on the Rota rod for 3 min were considered as passed to test⁶.

Effect of Locomotor activity in mice:

Locomotor activity was recorded with a digital activity cage. The animals were randomly divided into 3 groups and each mouse was individually placed in the actophotometer for 10 min to score the basal reading. All the animals were treated with vehicle, extract dose of 300mg/kg and standard drug diazepam (2mg/kg i.p.). After 30 min they were again placed individually into actophotometer to score locomotor activity. Mean changes in the locomotor activity was calculated for each group⁷.

Elevated plus maze test in mice:

This test is used as a standard model to assess anxiolytic activity of drugs. Locally fabricated apparatus consisting of two open arms (16×5cm) and two enclosed arms (16×5×12cm) evaluated to the height of 25cm (19-20) as validated by Lister, 1987 was used. Animals of all 3 groups were treated with vehicle, methanolic extract of *solanum pubescens* and reference standard diazepam (2mg/kg i.p.) respectively, 30 min before the test. The mice were placed individually in the centre of the maze, head facing towards open arm. The number of entries in open and closed arm and total time spent in open and closed arms respectively were recorded for a period of 5min. Entry into an arm was defined as to point when the animal places all four paws onto the arm^{8,9}.

Stair case:

The stair case test has become established for measurement of size specific deficits in coordinated paw reaching in mice, and has been shown to reveal impairments on the contra lateral side following unilateral lesions in a wide range of motor structures of the brain.

For experiments with mice, a stair case is composed of five identical steps of 2.5cm height 20cm wide and 7.5cm deep. The internal height of wall is constant along the stair case. Male mice with a weight between 18-24g are used. Each animal is used only once. The standard drug diazepam is administered orally 1 hr or 30 min subcutaneously before the test.

The animal is placed on the floor of the box with its back on the stair case. The number of steps climbed and the number of rears are counted over a 3 min period. A step is considered to be climbed only if the mouse has placed all four paws on the step. In order to simplify the observation the number of steps descended is not taken into account. After each test the box has to be cleaned in order to eliminate any olfactory cues which might modify the behavior of next animal.

III. Results:

Table.no.1: Effect of methanol extract of *Solanum pubescens* on exploratory behavior (Y-maze test) in mice.

EXPERIMENT	DOSE	NUMBER OF ENTRIES AFTER TREATMENT(min)			
		30	60	90	120
Control	5ml/kg	5.2±0.39*	5.3±0.43*	5.3±0.43*	5.3±0.49*
Diazepam	5mg/kg	3.2±0.28*	3.3±0.12*	3.5±0.23*	3.4±0.26*
MESP	300MG/KG	3.7±0.31*	3.7±0.27*	3.9±0.27*	3.9±0.31*

Values are the number of entries in 3 min (Mean±SEM n=6). *Significant difference between control group and treated group; p<0.05, ANOVA followed by Dunnett's multiple comparison test.

Table.no.2: Effect of Methanolic extract of *Solanum pubescens* on locomotor activity in mice.

S.no	Experiment	Locomotor activity (10min)		% change in activity
		Before treatment	After treatment	
1	Control	207.52±19.50	201.0 ± 9.44	-
2	Standard	214.83±22.48*	39.67±14.688*	84.29*
3	MESP	230.30± 23.59	97.16± 16.55*	67.41

Values are the percentage animals showing a negative results in 3 min (Mean±SEM, n=6). Significant difference between control group and treated group, *p< 0.001, ANOVA followed by Dunnett's multiple comparison test.

Table.no.3: Effect of Methanolic extract of *Solanum pubescens* on exploratory behavior (Head dip test) and Muscle relaxant activity (Traction test and Rota rod test).

S.no	Experiment	Head dip test	Traction test	Rota rod test	
				Fall off time in sec	
				Basal reaction time	After drug treatment
1	Control	95±8.4	0	29.28	31.32±3.78***
2	Standard	28±2.3*	100	31.50	7.54±3.71 (76.06)
3	MESP	30±2.8	80*	24.15	11.52±2.74** (52.29)

Values are the number of head dips in 3min (Mean±SEM, n=8). Significant difference between control and treated group; *p<0.05, **p<0.01, ***p<0.001, ANOVA followed by Dunnett's multiple comparison test.

Table.no.4: Effect of methanolic extract of *Solanum pubescens* on Central nervous system activity by Elevated plus maze test in mice.

Groups	30min				60 min				90min			
	No. entries into closed arm	Time spent in closed arm	No. entries into open arm	Time spent in open arm	No. entries into closed arm	Time spent in closed arm	No. of entries into open arm	Time spent in open arm	No. entries into closed arm	Time spent in closed arm	No. of entries in Open arm	Time spent in open arm
Control	6.3±1.36	3.18±0.27	4.5±0.76	1.56±0.39	2.16±0.47	4.51±0.12	0.5±0.22	0.02±0.01	6.5±0.76	3.77±0.22	2.83±0.60	0.22±0.04
Standard(2mg/kg)	1.83±0.47	2.8±0.61	0.66±0.33	0.06±0.03	0.5±0.22	2.12±0.95	0.66±0.21	0.05±0.03	6.66±1.38	3.61±0.17	2.66±1.11	0.15±0.02
<i>Solanum pubescens</i> (300 mg/kg)	4.83±1.40	2.52±0.72	1.33±0.61	0.06±0.03	0.83±0.16	4.43±0.05	0.03±0.21	0.04±0.02	7.16±0.94	2.39±0.25	1.83±0.30	0.14±0.03

Values are the number of entries into open and closed arms in 5min (Mean±SEM, n=6) Significant difference between control and treated group; *p<0.01, **p<0.02, ***p<0.05, ****p<0.001, ANOVA followed by dunnett's multiple comparison test.

Table.no.5: Effect of Methanolic extract of *Solanum pubescens* on Central Nervous System activity by stair case model in mice.

Groups	30 min		60 min		90 min	
	No. of rearings in 3min	No. of climbings in 3min	No. of rearings in 3min	No. of climbings in 3min	No. of rearings in 3min	No. of climbings in 3min
Control	19.83±2.28	3.33±1.23	16.16±2.49	1.83±1.08	16.16±0.98	3.5±1.06
Standard (2mg/kg)	5.66±1.23	4.66±0.88	4.5±0.72	1.83±0.47	5.83±2.33	2.66±1.14
<i>Solanum pubescens</i> (300mg/kg)	7.5±1.18****	0.83±0.40	8.17±1.85*	1.16±0.62	4.67±1.48****	1.5±0.76

Values are the number of steps climbed in 3 min (Mean±SEM,n=). Significant difference between control and treated group;*p<0.01, **p<0.02, ***p<0.05, ****p<0.001, ANOVA followed by Dunnett's multiple comparison test.

IV. Discussion:

In the present study, the effect of methanolic extract of *Solanum pubescens* on CNS activity has been evaluated. The result indicated that the methanol extract of *Solanum pubescens* influence the general behavioral profiles, as evidenced in the spontaneous activity, righting reflex, pinna reflex, grip strength and pain responses. Reduction of awareness and depressant action may be due to the action of the extract on CNS. Reduction of pinna reflex may be due to blocking synapse of the afferent pathway.

The myorelaxant effect was observed only with the higher dose of methanol extract of *Solanum pubescens* which resulted in an increase in the number of falls and a decrease in the time on the bar as detected by the Rotarod test. The intensity of reduction in exploratory behaviours in the treated animal groups which reflects the same line of action like the standard reference drug benzodiazepine, which acts as a anxiolytic (at low doses), anticonvulsants sedation and also produce and a myorelaxant effect at higher doses . The reduction in exploratory behavior in animals treated with methanol extract of *Solanum pubescens* is similar with the action of other CNS depressant agnts in Head dip test. A significant lack in motor coordination and muscle relaxant activity was also noted in animals treated with the extracts.

However, further investigation is underway to determine the exact phytoconstituents that are responsible for CNS depressant activity of methanol extract of *Solanum pubescens* and the receptors involved for the execution of the activity.

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