

## Research Article



## Effect of Methanolic Extract of *Canscora decussata* on Haloperidol – Induced Motor Deficits in Albino Mice

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### ABSTRACT

Use of antipsychotics like haloperidol in the treatment of schizophrenia and other affective disorders are known to produce extra pyramidal symptoms which are seen in Parkinson's disease. In the present study, an attempt has been made to evaluate the neuroprotective effect of methanolic extract of *Canscora decussata* on haloperidol (2mg/kg-intraperitoneal administration) induced catalepsy in mice by employing catatonial behavioural test. Behavioural changes caused by haloperidol were studied by rotarod test, grip strength test and locomotor activity by Actophotometer. In addition, antioxidant enzymes were also estimated to know the neurotoxic effect of haloperidol. All these assessments were done on 24mice which were divided into 4 groups (n=6). MECD was administered at 100mg/kg and 200mg/kg doses, 30min prior to haloperidol treatment for 7days. MECD significantly (\*P<0.05, \*\*P<0.01) improved the behavioural activities and striatal antioxidant status in a dose dependent manner. The results indicated that MECD has a protective role against catalepsy and behavioural changes induced by haloperidol and also possess antioxidant capacity.

**Keywords:** *Canscora decussata*, Haloperidol, Catalepsy, Neuroprotective.

### INTRODUCTION

A decrease in the dopamine (DA) innervations of the striatum due to loss of DA neurons in the substantia nigra is responsible for the core motor symptoms of Parkinson's disease (PD)<sup>1</sup>. The events which trigger and mediate the loss of nigral DA neurons is still unclear. Catalepsy induced by neuroleptics such as haloperidol has been used as an animal model for screening drugs for parkinsonism<sup>2</sup>. Catalepsy is defined as the failure to correct an externally imposed posture. Haloperidol reversibly blocks dopamine D<sub>2</sub> receptors and reduce dopaminergic transmission and thus produces extrapyramidal side effects<sup>3</sup>. GABA deficiency<sup>4</sup> and cholinergic dysfunction are proved to be cause for catalepsy.

*Canscora decussata* (Shankapushpi) has been used as a nervine tonic in ayurvedic medicine. Earlier studies has shown that it possess Anticonvulsant, Monoamino oxidase inhibiting<sup>5</sup>, Spermicidal, Anti inflammatory<sup>6</sup>, Anti-mycobacterium tuberculosis properties. The methanolic extract of this whole plant has been confirmed for its anticonvulsant activity<sup>7</sup>. Previously and now it was used to find its role in enhancing the dopaminergic transmission and antioxidant capacity in parkinson's disease.

### MATERIALS AND METHODS

#### Animals

Twenty four male swiss strain adult albino mice (*Mus musculus*), weighing 25-35 g were procured from King's Institute, Guindy. The animals were maintained in the animal house under standard laboratory conditions with natural dark and light cycle (approximately 12 h light / 12

h dark cycle) and room temperature (27±1°C) and constant humidity (60%) in accordance with Institutional Ethical Committee rules and regulations. They were fed on a standard balanced diet and provided with water *ad libitum*. Animals were acclimatized to laboratory conditions for 10days prior to the initiation of the experiment. The project proposal was approved by Institutional Animal Ethical Committee (IAEC 95/2009).

#### Collection of plant and Preparation of extract

The whole plant parts of *Canscora decussata* were used in this study. The plants were collected from Thirunalveli, Tamilnadu and authentication (Voucher specimen number-PARC/2010/500) was done by Prof. P. Jayaraman, PhD, Plant Anatomy Research Centre, Medicinal plant Research Unit, Tambaram, Chennai-45. The collected Plants were cleaned, air dried at room temperature and ground in to a fine powder with an automix blender and stored in a deep freezer until the time for use. The dried powder was defatted using Petroleum ether for a period of 24 hours. The powder was then dried and extracted by using Methanol as solvent in Soxhlet apparatus for 72 hours, at a temperature of 45°C. The extract (MECD) was concentrated to dryness under reduced pressure and controlled temperature (40–50°C) by using Rotary evaporator.

#### Drug treatment and Experimental design

The animals were divided into four groups, consisting of six in each group. Group I is Normal Control and received 10% Tween 80 (p.o). Group II is treated with Haloperidol (2mg/kg, i.p) (*SERENACE-RPG* Life Sciences Ltd.). Groups III and IV received 100mg/kg and 200mg/kg MECD (p.o)



respectively 30 minutes prior to haloperidol injection for 7 days.

At the end of the treatment after 7 days, catatonia behavioural study, rotarod test, grip strength test and locomotor activity test were performed for all 4 groups. Then the animals were sacrificed by cervical decapitation and the brains were excised and the striatum was separated and homogenized in ice cold phosphate buffer saline solution and used for biochemical assessments.

### Catalepsy behavioural test

Catatonias were measured by placing the animals on a flat horizontal surface with both hind limbs on a square wooden block (3 cm height), latency to move was calculated in seconds. The stages of catatonia induced with haloperidol were studied at 30, 60, 90 and 120 minutes after the administration of the plant extract (MECD). In stage I, the animal has no desire to make any movements; it sits quietly where it has been placed. However, a light push against the animal can elicit brief movements (score-0). In stage II, the animal remains as in stage I, but a push no longer elicits movements (score-0.5). In stage III, the animal assumes postures when its forelimb is placed on a block 3 cm high (score-1). In stage IV, the animal maintains its fixed position when, while sitting on its hind limbs, one of its forelimbs is placed on a block of 9 cm high and the other forelimb is allowed to hang free (score-2)<sup>7</sup>.

### Rotarod test

Motor coordination was measured on the seventh day using an automated rotarod (Inco). The animals were exposed to 10 trials on a rotating rod at 10 rpm at 5 minutes interval. The control mouse is able to remain on the rod for longer than 180 seconds. The treated mice were placed on the rod at intervals, and the time of the fall from the rod was noted. The test is terminated at 3 minutes<sup>8</sup>.

### Grip strength test

Male mice with an average weight of 18-30 g were used. In a preliminary experiment the animals were tested for their normal reactivity. The animals were exposed to a horizontal thin metallic wire suspended about 30 cm into the air which they immediately grasp with the forepaws. The mouse were released to hang on with its forelimbs. Normal animals are able to catch the metallic wire with the hind limbs and to climb up within 5 s. Only animals which fulfil this criterion were included into the experiment. After oral or subcutaneous administration the animals were tested every 15 min. Animals which are not able to touch the threat with the hind limbs within 5 s or fall off from the metallic wire are considered to be impaired<sup>9</sup>.

### Locomotor Activity using Actophotometer

The locomotor activity was measured using an Actophotometer (Inco). The movement of the animal in

the activity cage interrupts a beam of light falling on a photocell, at which a count was recorded and displayed digitally. Each mouse was placed individually in the Actophotometer for 10 minutes and the basal activity score was obtained. 30 min and 60 min after the oral administration of the vehicle or Standard or extract each mouse was retested for activity for 10 min. The difference in the activity was recorded considering before treatment values and after vehicle or standard or extract treatment values. Finally percentage decrease in the locomotor activity was calculated<sup>10</sup>.

### Assessment of Antioxidant status

#### Assay of Superoxide dismutase (SOD)

SOD was assessed by the inhibition of formation of NADH-phenazine methosulphate nitroblue tetrazolium formazan. The reaction was initiated by the addition of NADH after incubation for 90 s and stopped by the addition of glacial acetic acid. The colour formed at the end of the reaction was extracted in to the butanol layer and measured at 520 nm<sup>11</sup>.

#### Estimation of Lipid peroxidation products

One milliliter of suspension medium will be taken from 10% of the tissue homogenate. To this; 1 ml of 30% TCA will be added, followed by 1 ml of 0.8% TBA reagent. The tubes were covered with aluminum foil and kept in a shaking water bath for 30 minutes at 80°C. These were then centrifuged at 3000 rpm for 15 minutes. The absorbance of the supernatant was read at 535 nm at room temperature against the blank<sup>12</sup>.

#### Assay of Catalase

The tissue was homogenated in isotonic buffer (pH -7.4), the homogenate was centrifuged at 1000 × g for 10 minutes.

20 μl of 100-fold diluted tissue supernatant was added to 980 μl of the assay mixture. Assay mixture consists of 900 μl of 10 m mol/L of H<sub>2</sub>O<sub>2</sub>, 50 μl of Tris HCl buffer (pH-8) and 30 μl of distilled water.

The rate of decomposition of H<sub>2</sub>O<sub>2</sub> was monitored spectrophotometrically at 240 nm<sup>13</sup>.

CAT activity is expressed as K/mg protein, where 'K' is the first order rate constant.

#### Assay of Glutathione peroxidase (GPx)

The reaction mixture consisting of 0.2 ml of each EDTA, sodium azide and H<sub>2</sub>O<sub>2</sub>, 0.1 ml of suitably diluted tissue was incubated at 37°C at different time intervals.

The reaction was arrested by the addition of 0.5 ml of TCA and the tubes were centrifuged at 2000 rpm. To 0.5 ml of supernatant, 4 ml of disodium hydrogen phosphate and 0.5 ml DTNB was added and the colour developed was read out at 420 nm spectrophotometrically<sup>14</sup>.

Activity is expressed as μ moles of glutathione oxidized/minutes/mg protein



### Estimation of Reduced glutathione (GSH)

To 2ml of the homogenate, prepared in Kcl solution, 2.5 ml of 0.02 M EDTA was added and shaken. To 2ml of the mixture 4ml of cold distilled water and 1ml of 50% TCA was added and shaken for 10 minutes. The contents were centrifuged at 3000rpm for 15 minutes. 2 ml of the supernatant was mixed with 4ml of 0.4M tris buffer (ph 8.9). The whole solution was mixed well and 0.1ml of 0.01 M DTNB will be added, the absorbance was read within 5minutes of addition of DTNB at 412nm against reagent blank with no homogenated. For blank readings, the homogenate was substituted by 2 ml of distilled water.

Total glutathione will be calculated using the formula:

$$C_o = (A * D) / E$$

Where A is absorbance at 412nm, D is dilution factor, and E is the molar extinction coefficient ( $C = 13000M^{-1}CM^{-1}$ );  $C_o$  is the concentration of GSH<sup>12</sup>.

### Statistical Analysis

One way analysis of variance (ANOVA) followed by Dunnet's test were employed for the analysis of catalepsy, biochemical and other behavioural parameters.  $P < 0.01$ ,  $P < 0.05$  were considered significant.

### RESULTS

The cataleptic score was increased in haloperidol group after 60 and 90 minutes of administration. The score was significantly reduced after 60 minutes with the test drug MECD at both doses tested (100mg/kg and 200mg/kg)

(Table:1).

This reduction was significant through the period of observation, till 120 minutes. Maximum reduction in cataleptic activity was seen in 200mg/kg treated group out the observation period.

Table:2 shows the results of Rotarod test. In the haloperidol group, the retention time was reduced when compared to the normal control group and it was significantly improved in MECD treated groups. Among these two groups, maximum increase in the retention time was noted in 200mg/kg group.

Table:3 shows the results of Grip strength test. The fall off-time is reduced in the haloperidol group and it was improved by MECD treatment.

Table:4 shows the results of Locomotor activity using Actophotometer. In this test, MECD treated groups showed increased photocell count which was decreased significantly in haloperidol group than the normal control group count. The photocell count was significantly more in 200mg/kg MECD treated group.

Striatal TBARS and antioxidant status are presented in Table:5. The striatal TBARS was significantly elevated in haloperidol treated group of mice when compared to control group. MECD reduced the TBARS level. SOD, CAT, GPx and GSH activities were significantly decreased in haloperidol treated group when compared to control group and were again restored significantly on treatment with MECD at 100mg/kg and 200mg/kg doses.

**Table 1:** Effect of MECD on Catalepsy behavioral test

Group (n=6)	Dose (kg)	Cataleptic scores at different time points(min)			
		30	60	90	120
I Control	10% Tween80	14.81±0.83 <sup>*</sup>	16.32±0.07 <sup>**</sup>	16.18±0.01 <sup>**</sup>	15.36±0.04 <sup>**</sup>
II Haloperidol	2mg	11.37±1.25	23.6±1.8	33.1±3.62	46.3±4.47
III MECD + Haloperidol	100mg+2mg	12.92±0.76 <sup>**</sup>	13.18±0.01 <sup>**</sup>	13.92±4.12 <sup>*</sup>	11.21±3.97 <sup>**</sup>
IV MECD + Haloperidol	200mg+2mg	7.21±0.65 <sup>*</sup>	9.32±0.31 <sup>**</sup>	8.1±0.16 <sup>*</sup>	8.23±0.03 <sup>**</sup>

Values are mean±SEM; n=6 in each group. ANOVA followed by Dunnet's test. Compared with Haloperidol group, <sup>\*</sup> $P < 0.05$ , <sup>\*\*</sup> $P < 0.01$ .

**Table 2:** Effect of MECD on Rotarod test

Group (n=6)	Dose (/kg)	Retention time(sec)
I Control	10% Tween80	96.01±2.13 <sup>**</sup>
II Haloperidol	2mg	52.16±3.17
III 100mg/kg MECD + Haloperidol	100mg+2mg	72.76±5.79 <sup>**</sup>
IV 200mg/kg MECD+ Haloperidol	200mg+2mg	89.32±4.19 <sup>**</sup>

Values are mean±SEM; n=6 in each group. ANOVA followed by Dunnet's test. Compared with Haloperidol group, <sup>\*\*</sup> $P < 0.01$ .



**Table 3:** Effect of MECD on Grip-strength test

Group (n=6)	Dose (/kg)	Fall-off time(sec)
I Control	10% Tween 80	89.5±0.002**
II Haloperidol	2mg	42.1±0.001
III 100mg/kg MECD + Haloperidol	100mg+2mg	59.8±0.005**
IV 200mg/kg MECD+ Haloperidol	200mg+2mg	72.6±0.004**

Values are mean±SEM; n=6 in each group. ANOVA followed by Dunnett's test. Compared with Haloperidol group, \*\*P<0.01.

**Table 4:** Effect of MECD on Actophotometer

Group (n=6)	Dose (/kg)	Photocells count			
		30min	%Inhibition	60min	%Inhibition
I Control	-	292.5±15.31**	-	276.0±11.39**	-
II Haloperidol	2mg	83.6±14.49	72.60	79.2±8.68	76.81
III 100mg/kg MECD + Haloperidol	100mg+2mg	154.5±17.54*	62.23	137.3±16.21*	72.18
IV 200mg/kg MECD + Haloperidol	200mg+2mg	186.4±20.52**	53.12	176.9±20.13**	51.29

Values are mean±SEM; n=6 in each group. ANOVA followed by Dunnett's test. Compared with Haloperidol group, \*P<0.05, \*\*P<0.01

**Table 5:** Effect of MECD on antioxidant status in Haloperidol treated mice

Groups (n=6)	Dose (/kg)	SOD (μ/mg protein)	LPO (n moles/mg)	CAT (μ/mg protein)	GPx (μ/mg protein)	GSH (μ/mg protein)
I Control	-	5.18±0.81**	2.2±0.38**	0.56±0.01**	11.87±0.16**	0.58±0.01**
II Haloperidol	2mg	2.19±0.16	5.13±0.22	0.16±0.01	7.3±0.02	0.21±0.06
III MECD + Haloperidol	100mg+2mg	3.90±0.26*	4.06±0.06**	0.28±0.01**	8.13±0.04**	0.38±0.02**
IV MECD + Haloperidol	200mg+2mg	4.82±0.18**	3.83±0.11*	0.43±0.01**	9.36±0.04**	0.46±0.04**

Values are mean±SEM; n=6 in each group. ANOVA followed by Dunnett's test. Compared with Haloperidol group, \*P<0.05, \*\*P<0.01.

## DISCUSSION AND CONCLUSION

Neuroleptics such as haloperidol acts by blocking dopamine D<sub>2</sub> receptors and controls the positive symptoms such as delusions, hallucinations and thought disorders in schizophrenia, but at the same time produces some extrapyramidal symptoms as side effects. These neuroleptic-induced extrapyramidal symptoms are similar to motor deficits in Parkinson's disease<sup>15</sup>.

Haloperidol-induced catalepsy in rodents has been used as an animal model for screening drugs for PD, which has been employed in the present study. It is also an important animal model to test for extrapyramidal side effects of antipsychotic agents<sup>16</sup>. Evidences indicates that drugs which potentiate or attenuate neuroleptic induced catalepsy in rodents might aggravate or reduce the extrapyramidal side effects respectively<sup>2</sup>.

In our study, Methanolic extract of *Canscora decussate* (MECD) administered in 100mg/kg and 200mg/kg doses in mice showed a significant reduction in cataleptic scores.

Especially, reduction was greater at the higher doses (200mg/kg) of administration. Even in the present study,

this test provided a required evidence about the anti-parkinsonism activity of the plant extract.

Haloperidol treated mice when subjected to motor integration tests such as Rotarod test and Grip strength test, showed a decrease in muscle coordination which could be due to a loss of muscular strength. Treatment with MECD showed a significant improvement in the muscle coordination as there is an increase in retention time and fall-off time in rotarod and grip strength test respectively. Locomotor activity was also studied using Actophotometer in which MECD improved the photocells count which was significantly less in haloperidol treated group.

Many clinical and preclinical studies have suggested the involvement of reactive oxygen species in haloperidol induced catalepsy<sup>17</sup>. In the present study, the haloperidol treated group showed an increase in oxidative stress when compared to normal control group. Haloperidol treatment significantly increased TBARS levels in lipid peroxidation which was reversed by MECD treatment. Also, Haloperidol decreased SOD, Catalase and brain glutathione levels than the normal range. These levels were significantly protected on MECD treatment, especially at dose of 200mg/kg. Unlike idiopathic

parkinsonism, Striatal content of dopamine is not reduced by administration of butyrophenones and phenothiazines<sup>18</sup>.

This study provides an evidence that *Canscora decussata* has a neuroprotective action against haloperidol induced catalepsy which suggests that this plant extract can be usefully employed in the management of PD, but still needs exploration.

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