Indian Journal of Experimental Biology
Vol. 44, January 2006, pp. 45-48

Effect of BR-16A (Mentat®), a polyherbal formulation on drug-induced catalepsy in mice

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Received 31 January 2005; revised 15 September 2005

Parkinson's disease (PD) is a neurodegenerative disease characterized by the selective loss of dopamine (DA) neurons of the substantia nigra pars compacta (SNc). The events, which trigger and/or mediate the loss of nigral DA neurons, however, remain unclear. Neuroleptic-induced catalepsy has long been used as an animal model for screening drugs for Parkinsonism. Administration of haloperidol (1 mg/kg, ip) or reserpine (2 mg/kg, ip) significantly induced catalepsy in mice. BR-16A (50 and 100 mg/kg, po), a polyherbal formulation or ashwagandha (50 and 100 mg/kg, po), significantly reversed the haloperidol or reserpine-induced catalepsy. The results indicate that BR-16A or ashwagandha has protective effect against haloperidol or reserpine-induced catalepsy and provide hope that BR-16A could be used in preventing the drug-induced extrapyramidal side effects and may offer a new therapeutic approach to the treatment of Parkinson's disease.

Keywords: Ashwagandha, BR-16A, Catalepsy, Haloperidol, Reserpine
[**IPC Code**: Int Cl[7] A61 P]

Neuroleptics are extensively used in the treatment of schizophrenia and other affective disorders[1]. Unfortunately their use is often associated with distressing side effects involving in the case Parkinson's and tardive dyskinesia[2,3]. Neuroleptic-induced catalepsy has long been used as a model for the extrapyramidal side effects (EPS), such as Parkinsonian-like bradykinesia associated with antipsychotic use in humans[4]. Evidences indicate that haloperidol or reserpine induce catalepsy in animals and this behavioral response has long been used as a model for extra pyramidal side effects[5,6]. Besides, dopamine receptor blockade and catecholamine depletion, other neurochemical hypotheses have been proposed for the development of catalepsy such as striatonigral GABAergic, cholinergic, glutamate and sertonoergic etc [7-9].

BR-16A, an herbal psychotropic preparation contains the following indigenous ingredients: Brahmi (*Bacopa monnieri*), Mandakparani (*Centella asiatica*), Vacha (*Acorus calamus*), ashwagandha (*Withania somnifera*), Giloi (*Tinospora cordifolia*), Amla (*Embelica officinalis*), Shankhpushpi (*Evolvulus aisinoides*), Kuth (*Saussurea lappa*) and Triphala (*Terminalia belerica, Terminalia chebula* and *Terminalia arjuna*)[10]. BR-16A has been reported to be effective in improving ability and behavioral disturbances in mentally retarded children[10]. It is also reported to be beneficial in cerebral deficit, behavioral disturbances following postnatal organic lesions of CNS[11]. Ashwagandha, a major constituent of BR16A formulation is used in Ayurvedic medicine to (a) attenuate cerebral functional deficits in the geriatric population, (b) augment the faculty of learning and memory retention in both normal and deficient individuals and (c) provide non-specific host defense.

A decoction containing cows milk powdered *Mucuna pruriens* seeds and *Withania somnifera* has been reported to be effective in 18 clinically diagnosed parkinsonian patients[12] and tardive dyskinesia management therapy[13]. Although the exact mechanism(s) of action for these properties are not fully understood, *Ashwagandha* is reported to influence various neurotransmitter receptors in the CNS. Further BR-16A and Ashwaganda are reported to be safe in long-term use with no adverse effects[14,15].

With this background, the present study has been designed to explore the protective effect of BR-16A a polyherbal formulation on drug-induced catalepsy.

Materials and Methods

Animals—Laca mice of either sex (weighing 20-25 g), bred in Central Animal House facility of Panjab University, were used. The animals were housed

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under standard light/dark cycle with food and water provided *ad libitum.* Animals were acclimatized to laboratory condition before test. Each animal was used once. The experiments were performed between 0900-1600 hrs. The experimental protocols were approved by the Institutional Ethics Committee and were conducted according to the Indian National Science Academy Guidelines for the use and care of experimental animals.

Drugs, dosages and treatment schedule—Following drugs were used in the study at the dosages mentioned: haloperidol (1 mg/kg, ip, Searle, India), reserpine (2 mg/kg, ip, Loba Chemical, India), BR-16A (50 and 100 mg/kg, po, Himalayan Drug Company, Bangalore), and Ashwagandha root extract (50 and 100 mg/kg, po, Himalayan Drug Company, Banglore). Reserpine was dissolved in few drops of acetic acid and volume was made up with distilled water. BR-16A or ashwagandha was suspended in 0.5% of sodium carboxy-methyl-cellulose. Haloperidol was dissolved in distilled water. All drugs were administered in a constant volume of 1 ml/100 g body weight of mouse. Drugs were administered 30 min before haloperidol or reserpine administrations to overnight-fasted animals. Overnight fasted animals were used in order to increase the drug absorption of orally administered drugs.

Behavioral assessment—A cataleptic behaviour was measured with a high bar test method. Catalepsy score was measured for 4 hr at one-hour intervals after haloperidol or same cataleptic score after 4 hr in reserpine administration, by gently placing both the forepaw of the mouse over a metal bar (diameter 2-5 mm suspended 6 cm above the tabletop). The intensity of catalepsy was assessed by counting the time in seconds until the mouse brought both forepaws down to the tabletop, with a maximum cutoff time of 3 min. Finally, scores at different time points (0, 60, 120, 180 and 240 min after haloperidol injection) were added and expressed as cumulative catalepsy score for comparison purpose. Similarly, catalepsy score was measured 4 hr after reserpine administration in reserpine model. In all the experiments the scorer was blind to the treatment given to the mice.

Statistical analysis—Catalepsy data were expressed as mean ± SE and analyzed by one-way analysis of variance (ANOVA) followed by Dunnett test. In all the tests, the criterion for statistical significance was $P<0.05$.

Results

Effect of BR-16A or Ashwagandha on haloperidol induced catalepsy—In the present study haloperidol produced a time dependent increase in cataleptic state, which was significant as compared to vehicle treated animals (Fig.1). BR-16A (50 and 100 mg/kg, po), significantly reduced severity of haloperidol-induced catalepsy at all time intervals (Fig. 2). Ashwagandha (50 and 100 mg/kg, po) significantly and dose dependently reduced cataleptic score as compared to haloperidol alone treated animals at 60, 120 and 180 min (Fig. 3). BR-16A or Ashwagandha reduced the cumulative catalepsy score as compared to haloperidol treated animal (Table 1).

Effect of BR-16A or Ashwagandha on reserpine-induced catalepsy—Reserpine (2 mg/kg, ip) produced significant catalepsy as compared to vehicle treated

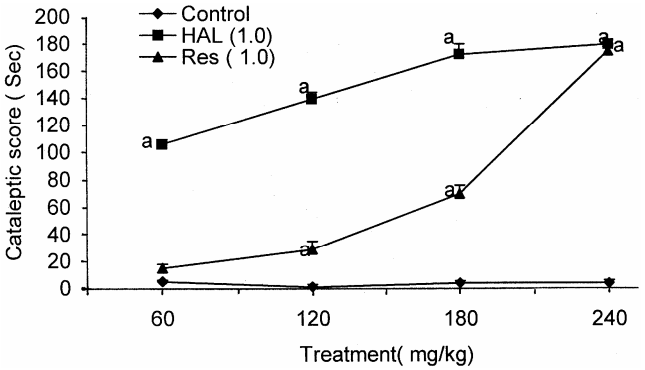

Fig. 1—Effect of haloperidol (HAL) and reserpine (RES) on cataleptic behaviour in mice. [Values are mean ± SE [a]$P<0.05$ as compared to vehicle treated group (ANOVA followed by Dunnett's test]

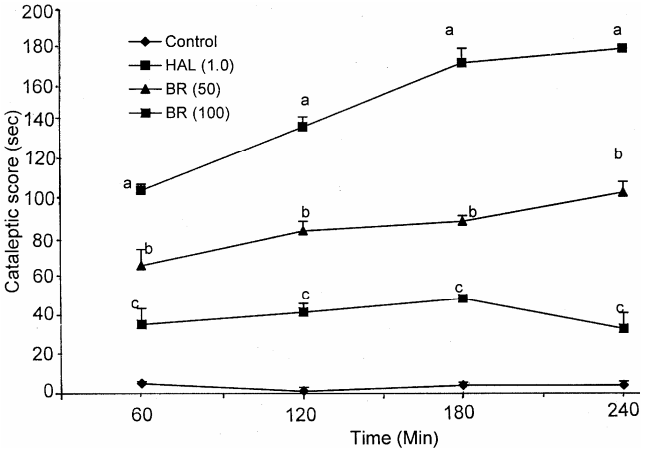

Fig. 2—Effect of BR-16A treatment on haloperidol induced catalepsy in mice. Values are mean ± SE P values: <0.05 as compared to [a]vehicle treated group; [b]haloperidol (10 mg/kg); [c]BR16A (50 mg/kg) ANOVA followed by Dunnett test

group (P<0.05). BR 16A or Aswagandha dose dependently (50, 100 mg/kg, po) reduced the severity of reserpine-induced catalepsy (Table 2).

Discussion

Haloperidol-induced catalepsy is one of animal models to test for the extrapyrimidal side effects of antipsychotic drugs[16]. The haloperidol, (a non-selective D_2 dopamine antagonist)-induced catalepsy is primarily due to blockade of dopamine receptors in the striatum. Serotonin is also involved in neuroleptic catalepsy[17].

Protective effect of BR-16A (50 and 100 mg/kg) or Ashwagandha (50 and 100 mg/kg) against haloperidol (1 mg/kg) or the vesicular monoamine transporter blocker, reserpine (2 mg/kg)-induced catalepsy suggests that these herbal drugs influence not only dopamine receptor-mediated neurotransmission but also serotonergic receptor-mediated neurotransmission. The results are also consistent with the earlier study reporting valuable effect of Ashwagandha against haloperidol-induced tardive dyskinesia[13].

Further, Ashwagandha, which is one of the ingredients of the BR-16A formulation, also reduced the severity of both haloperidol and reserpine-induced catalepsy score. Ashwagandha has been proved to be neuroprotective in stress-induced dopamine neuron degeneration[18]. It has been shown to be a free radical scavenger and inhibited lipid peroxidation in the central nervous system[19].

The above observations indicate that anticataleptic effect of BR-16A may be due to Ashwagandha present in the formulation. However role of other ingredient present in the BR-16A formulation cannot be ignored and required to be investigated.

Table 1—Effect of Ashwagandha *(ASH)* or BR-16A on cumulative catalepsy score(s) in haloperidol (HAL) treated animals
[Values expressed in min are mean ± SE of 5 animals in each group]

Group no.	Treatment, (mg/kg)	Cumulative catalepsy (sec)
1	Vehicle	23.0 ±1.4
2	HAL (1)	597.7±6.9 [a]
3	ASH (50)	426.33±8.1 [b]
4	ASH (100)	377.0±9.2 [b,c]
5	BR (50)	204.6±5.4 [b]
6	BR (100)	160.2±3.5 [b,d]

P values: < 0.05 as compared to
[a]vehicle treated group; [b]haloperidol (10 mg/kg),
[b]Ashwagandha (50 mg/kg), [d]BR16A (50 mg/kg)
ANOVA followed by Dunnett's test

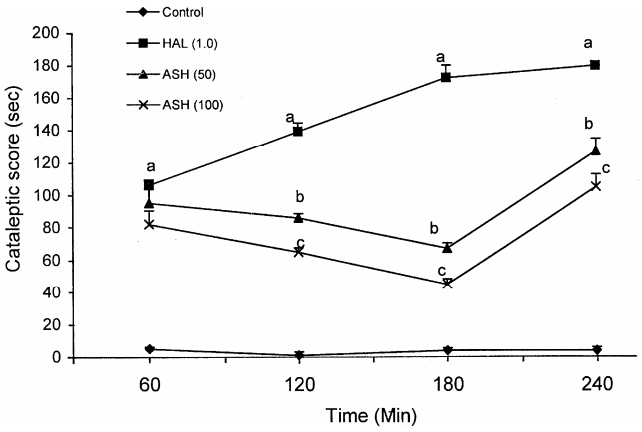

Fig. 3—Effect of Ashwagandha (ASH) on haloperidol induced catalepsy. Values are mean ± S.E. *P* values: <0.05 as compared to [a]vehicle treated group; [b]haloperidol (10 mg/kg); [c]ASH (50 mg/kg) ANOVA followed by Dunnett test

Table 2—Effect of Ashwagandha *(ASH)* or BR-16A on reserpine (RES) induced catalepsy
[Values expressed in min are mean ± SE of 5 animals in each group]

Group no	Treatment, (mg/kg)	Catalepsy score(s) after 4hr
1	Control	3.5±2.3
2	RES (2)	180±0.0 [a]
3	ASH (50)	44.8±2.8 [b]
4	ASH (100)	31.3±1.2 [b, c]
5	BR (50)	40.9 ±3.0 [b]
6	BR (100)	28.48±3.9 [b,d]

P values: < 0.05 as compared to
[a)]vehicle treated group; [b)] RES (2 mg/kg),
[c)]Ashwagandha (50 mg/kg), [d)] BR16A (50 mg/kg)
ANOVA followed by Dunnett's test

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