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Effect of various extracts of *Tabernaemontana divaricata* on haloperidol induced catalepsy in rats

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ABSTRACT

Parkinson's disease (PD) is one of the neurodegenerative diseases with selective loss of dopamine neurons of the substantia nigra pars compacta. In the present study, anti-cataleptic activity of *Tabernaemontana divaricata* leaves extracts *viz.* aqueous and ethanolic at different doses (50, 100 and 150 mg/kg i.p.) were studied using haloperidol (1 mg/kg, i.p.) induced catalepsy in rats which is a useful animal model for screening drugs for Parkinson's disease. Both the extracts were found to reduce catalepsy significantly ($P<0.001$) as compared to the haloperidol treated rats showing greater effect at 150 mg/kg i.p. dose. Thus the present study reveals the anti-cataleptic activity of *Tabernaemontana divaricata* evaluating the traditional folklore medicinal use of the plant.

Key Words: *Tabernaemontana divaricata*, Parkinson's disease (PD), catalepsy, haloperidol, bar test.

INTRODUCTION

Neurological disorders include brain injuries, neuro-infections, multiple sclerosis, Parkinson's disease, anxiety, depression, catalepsy etc. Among these anxiety and depression are the most common neurological disorders affecting up to one billion people worldwide as reported by World Health Organization (WHO) (Antony *et al.*, 2010).

Parkinson's disease (PD) is one of the neurodegenerative diseases with selective loss of dopamine neurons (DA) of the substantia nigra pars compacta (SNc). However, the events which trigger and mediate the loss of nigral DA neurons still remain unclear. Neuroleptic-induced catalepsy has been widely used as an animal model for screening antiparkinson drugs (Sanberg *et al.*, 1988). The blockade of DA transmission produces catalepsy (Sanberg, 1980; Marie-Louise *et al.*, 2001) in rats and extrapyramidal side effects in humans (Farde *et al.*, 1992) which has been evident in several studies.

Catalepsy is a condition in which the animal retains the imposed posture for long time before retrieval of the normal posture. Catalepsy is a sign of extrapyramidal effect of drugs. Haloperidol is widely and commonly prescribed typical anti-psychotic drug for the treatment of schizophrenia and other effective disorders (Seeman *et al.*, 1996; Kulkarni and Naidu, 2001) and can be used to induce catalepsy in animals.

It has been proposed that haloperidol induced oxidative stress causes due to generation of free radical catecholamine metabolism by monoamine oxidases (MAOs) (Mahdik and Mukherjee, 1996). Significant oxidative stress observed in the brain regions of animal with acute (Shivakumar and Ravindranath, 1992) and chronic (Shivakumar and Ravindranath, 1993) administration of haloperidol which is been demonstrated by loss of the nonprotein thiol anti-oxidant glutathione (GSH) and by the increase of the lipid peroxidation product, malonaldehyde. The pathophysiology of catalepsy still remains unclear. Theories implicating central cholinergic dysfunction, - γ-amino butyric acid (GABA) deficiency (Somani *et al.*, 1999), oxidative stress (Kedar, 2003) and 5 - hydroxy tryptamine (5-HT) (Silva *et al.*, 1995) dysfunction have been proposed. Haloperidol is a dopamine D2 receptor antagonist that reduces dopaminergic transmission in basal ganglion thus producing a state of catalepsy in animals (Seeman *et al.*, 1996) *Tabernaemomntana divaricata* L (Family:Apocynaceae) commonly called as Chandni in Hindi and crape jasmine in English, found in the dry regions of India, Sri Lanka and Bangladesh. Two types of Crape Jasmine are available, one type has a single blossom and the other has a clustered blossom. Both types have a white-colored flower. Height of the plant is generally 5 to 6 ft. The leaves are large, deep green in colour with shiny upper surface, about 6 inches in length and 2 inches in width. Small clusters of waxy blossoms grow on the stem tips (Van Beek *et al.*, 1984; Leeuwenberg, 1991).

The beneficial properties of *T. divaricata* are anti-infection, anti-tumor action and analgesia. Enhancing the cholinergic function may be therapeutically beneficial for many neurodegenerative diseases, specifically myasthenia gravis and Alzheimer's disease (Van Beek *et al.*, 1984). Therapeutically active constituents from *T. divaricata* include alkaloids, terpenoids, steroids, flavonoids, tannins, phenyl propanoids, phenolic acids etc.

MATERIALS AND METHODS

Plant material

The leaves of *Tabernaemontana divaricata* (TD) were collected in January, 2010, from Bhopal, M.P., India. The plant was identified and authenticated by Dr. D. V. Amla, Deputy Director, National Botanical Research Institute, Lucknow, India, and a voucher specimen no. Tit/NBRI/CIF/141/2009 was deposited in Department of Pharmacognosy and Phytochemistry, TIT-Pharmacy, Bhopal.

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Table 1: Comparative anticataleptic activity of various extracts of *Tabernaemontana divaricata* with the standard drug (L-Dopa).

Group	Treatment	Dose	Time in Minutes (Mean Catalepsy Time)						
			0 min	15 min	30 Min	45 Min	60 Min	75 Min	90 min
Group-I	Haloperidol	1mg/kg, i.p	0.0±0.0	0.64±0.16	1.71± 0.21	2.33±0.29	3.20± 0.59	4.02± 0.63	4.59± 0.59
Group-II	Haloperidol + L-Dopa	30 mg/kg, i.p	0.0±0.0 ns	0.0±0.0***	0.0±0.0***	0.0±0.0***	0.05±0.08 ***	0.10±0.08 ***	0.23±0.04 ***
Group-III	Haloperidol + aqueous extract	50 mg/kg, i.p	0.0±0.0ns	0.55±0.10 ns	1.57±0.10 ns	2.20±0.50 ns	2.77± 0.42 ns	2.84±0.44*	3.04± 0.20***
Group-IV		100 mg/kg, i.p	0.0±0.0 ns	0.46±0.14 ns	1.50±0.15ns	2.16±0.59ns	2.52± 0.60ns	2.73± 0.47**	2.83± 0.36***
Group-V		150mg/kg, i.p	0.0±0.0 ns	0.42± 0.14 ns	1.35±0.29 ns	1.47± 0.27 **	1.75± 0.61 ***	1.73± 0.60 ***	1.99± 0.53***
Group-VI	Haloperidol + ethanolic extract	50 mg/kg, i.p	0.0±0.0 ns	0.40±0.19 ns	1.54± 0.31ns	1.31±0.31 ***	1.78± 0.63 ***	1.84±0.55***	1.99± 0.54***
Group-VII		100 mg/kg, i.p	0.0±0 .0ns	0.34±0.13**	0.66±0.58 ***	0.86±0.48 ***	0.86± 0.48 ***	0.95± 0.44 ***	1.33± 0.46 ***
Group-VIII		150 mg/kg, i.p	0.0±0 .0ns	0.0±0.0 ***	0.0±0.0***	0.0±0.0 ***	0.19±0.21***	0.21± 0.19 ***	0.27± 0.15***

Values are expressed as mean±S.E.M. (n = 6). Values are statistically significant at *** P<0.001, ** P<0.01, *P<0.05, ns > 0.05 vs. control group respectively (One-way ANOVA followed by Tukey's post hoc test).

Preparation of extract
The leaves were dried in shade and stored at 25°C., powdered, passed through sieve no. 40. The dried powdered leaves of TD (500g) were first defatted with Petroleum Ether (60-80°C) and later extracted with ethanol and distilled water separately by maceration for 5 days. After completion of the extraction, the solvent was removed by distillation and concentrated *in vaccuo* (40°C) to yield ethanolic and aqueous extract respectively.

Preliminary phytochemical screening of TD
The preliminary phytochemical investigation was carried out with ethanolic and aqueous extracts of leaves of *T. divaricata* for qualitative identification of phytochemical constituents. Phytochemical tests were carried out by standard methods (Khandelwal, 2006; Kokate, 1994).

Animals
Male wistar rats weighing 200±20g were provided by the animal house of TIT Pharmacy, Bhopal, from the stock originally purchased from, National Institute of Nutrition, Hyderabad, India. Animals were made available with the standard animal feed and water supply ad libitum before the experiments. The animal studies were approved by the Institutional Animal Ethics Committee (Reg. no. 831/bc/04/CPCSEA), New Delhi, India. For each experimental study rats were starved for 18h with access to water only.

Drug and chemicals
Levodopa was obtained from Sun Pharma, and Haloperidol was procured from RVG Life Sciences Ltd.

Acute toxicity study
Acute toxicity study was carried out for the extracts of TD following Organization of economic co-operation and development (OECD) guidelines (OECD guideline, 2001). The extract was dissolved in distilled water in a dose of 2 g/kg body weight and orally administered to overnight-fasted, healthy rats (n = 6).The animals were observed continuously for 24 h for mortality.

Catalepsy bar test
The method described by Pemminati *et al.* (2007), was followed for the anticataleptic activity. The animals were divided into eight groups (n=6). Group I served as Control (Haloperidol 1mg/kg, i.p.), Group II as Standard (Levodopa 30 mg/kg i.p.), Group III-V served as the test group treated with aqueous extract (50, 100, 150 mg/kg, i.p., respectively) and Group VI-VIII treated with ethanolic extract (50, 100, 150 mg/kg, i.p. respectively). Standard bar test was used to measure the catalepsy. Catalepsy was induced by Haloperidol (1.0 mg/kg i.p.) and examine at every 15 minutes interval for 90 minutes. The duration for which the rat retains the forepaws extended and resting on the elevated bar (4 cm high and 1cm diameter) was considered as cataleptic score. The end point of catalepsy was considered when forepaws of the rat were removed from the bar or the rat moved its head in an exploratory manner. A cut-off time of 5 minutes was applied. All observations were made between 10.00 to 16.00 hrs in a quiet room at room temperature.

Statistical analysis
The results are expressed as mean ± S.E.M. Data were analyzed using one-way analysis of variance (ANOVA) after Tukey's multiple comparison tests. $P < 0.05$ was considered statistically significant in all the cases.

RESULTS

Preliminary phytochemical screening of TD
Phytochemical screening of TD revealed the presence of alkaloids, tannins, resins, proteins, amino acids, flavonoids, saponins, phenols, glycosides, steroids, triterpenoids, fixed oils and fats.

Catalepsy bar test
The cataleptic score was significantly reduced after 15 min, with all drugs *viz.* the standard drug Levodopa (30 mg/kg, i.p.), the aqueous extract of TD (50-150 mg/kg, i.p.) and the ethanolic extract of TD (50-150 mg/kg, i.p.). Ethanolic extract of TD significantly reduce the cataleptic score in a dose dependent manner. The reduction in cataleptic scores with ethanolic extract of TD at a dose of 150 mg/kg, i.p. was significant ($P < 0.001$) throughout the period of observations, except for 0 minutes. Both the extracts of TD *viz.* aqueous and ethanolic (50- 150 mg/kg, i.p) reduce cataleptic score significantly at 90 min. ($P < 0.001$).

DISCUSSION

In catalepsy we observe a failure to correct an externally imposed posture which is a measure of akinesia and can be evaluate using the bar test (Hubbard and Trugman, 1993). Postsynaptic striatal dopamine D1 and D2 receptors are blocked in case of a typical neuroleptic induced catalepsy (Sanberg, 1980; Farde *et al.*, 1992).

Despite the above evidence, role of several other neurotransmitters such as acetylcholine, GABA and serotonin, have also been phenomenon (Klemm, 1985; Somani *et al.*, 1999). Along with dysfunction of various neurotransmitters in haloperidol induced catalepsy, involvement of reactive oxygen species has also been suggested by many clinical and preclinical studies (Polydoro *et al.*, 2004; Sagara, 1998). The neuroleptic agents induce catalepsy by inhibiting dopamine D2 receptors in the substantia nigra (Ossowska *et al.*, 1990). Haloperidol block dopamine receptors present in the striatum and nucleus accumbens thus induce catalepsy.

Pathology of haloperidol induced catalepsy underlying increased oxidative stress. *Tabernaemontana divaricata* have anti- oxidative effects (Surya *et al.*, 2011), thus keeping the above facts in mind the present study was done to evaluate the anti-cataleptic effect of *Tabernaemontana divaricata* in haloperidol induced cataleptic rats.

In the present study, Haloperidol (1.0 mg/kg, i.p.) induced significant catalepsy in rats which is been apparent by a considerable augmentation of time spent on the bar as compared to the control group. Above study revealed that, both the extracts *viz.*; aqueous and ethanolic extract of TD protect rats from catalepsy as compared to the standard drug, Levodopa.

In the above study we observed that the various extracts reduce duration of catalepsy in rats. Phytochemical screening of TD revealed the presence of alkaloids, tannis, resins, proteins, amino acids, flavonoids, saponins, phenols, glycosides, steroids, triterpenoids, fixed oils and fats. Both the extracts of TD *viz.* aqueous and ethanolic (50- 150 mg/kg, i.p) reduce cataleptic score significantly at 90 min. ($P < 0.001$). The activities at a dose of 50 – 150 mg/kg i.p. are comparable with that of standard levodopa.

CONCLUSION

Our findings confirm the anticataleptic activity of both aqueous and ethanolic extracts of *Tabernaemontana divaricata*. From the study it may be concluded that the test drug can safely be replaced as an alternative agent in preventing and treating the extrapyramidal side effects of antipsychotic agents in clinical practice.

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