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Role of methanolic extract of *Hemidesmus indicus* in haloperidol-induced parkinsonism in albino mice-A preliminary study

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ABSTRACT

Objective: To evaluate & establish the antiparkinsonian activity of methanolic extract of *Hemidesmus indicus* (MEHI) against haloperidol-induced parkinsonism in *Swiss* albino mice.

Materials & Methods: In the present study the MEHI was prepared by successive solvent extraction (Soxhlet apparatus). Two doses i.e. 75 & 100 mg/kg of MEHI were evaluated for antiparkinsonian activity in haloperidol-induced parkinsonism (catalepsy) in albino mice. Benzhexol was used as standard drug (positive control).

Results: Pre-treatment of test animals with *Hemidesmus indicus* (75 & 100 mg/kg i.p) reduced the catalepsy score in haloperidol treated mice. Both the doses i.e. 75 & 100 mg/kg (i.p.) of *Hemidesmus indicus* showed protective effects against haloperidol-induced catalepsy.

Conclusion: The present study suggests that the *Hemidesmus indicus* has antiparkinsonian activity against haloperidol-induced parkinsonism in albino mice. Phenols and Tannins may be the responsible leads for the present study. Further, in-depth studies are needed to explore & establish the role of Terpenes and Phenolic content of *Hemidesmus indicus* for their antiparkinsonian mechanisms.

INTRODUCTION

Parkinsonism is an age-related second most common neurodegenerative disorder, affecting 1.4% of the population over the age of 55 years globally (Fatai, 2012; Oleg et al., 2013). An estimated 5 million people worldwide have parkinson's disease, with one million people each in the US and Europe (Fatai, 2012; Olanow and Koller, 1998). It is estimated that about 2% of people over 65 years of age and 4-5% of people are sufferers of parkinson's disease (Oleg et al., 2013). With the aging of the population and the significant increase in the number of vulnerable individuals older than 60 years, it is projected that the prevalence of parkinson's disease will increase dramatically in the coming decades (Fatai, 2012; Olanow and Koller, 1998). Parkinson's disease was first described by James Parkinson in 1817. He described this neurological disorder as shaking palsy and wrote a monograph entitled "An essay on the shaking palsy" (Fatai, 2012; Satoskar et al., 2009). It is slowly progressing neurodegenerative disorder that impairs the quality of life of patients and leads to increased health-care costs. It is characterized by tremor, muscular rigidity, sudden loss of postural reflexes, akinesia & or unstable posture (catalepsy) (Michalowaka et al., 2005). In addition disturbances of equilibrium and autonomic functions frequently occur. These symptoms of parkinsonism are attributed to low levels of dopamine in the fore brain area (Fatai, 2012; Olanow and Koller, 1998; Klopman and Aleksandr, 2002). Levodopa, a dopamine structural analog which improves the level of dopamine in brain, is the best presently available medication for the treatment of parkinson's disease. Unfortunately, on long term use it produces unwanted effects (Klopman and Aleksandr, 2002). Dopamine agonists are other alternatives which can be employed initially to delay the onset of motor complications but they are unable to control motor symptoms, incidences of dopaminergic adverse events are more, and they are expensive (Carl, 2004).

The use of herbs, herbal extracts or plant derived pure chemicals to treat disease is a therapeutic modality, which has gained immense popularity. At present there is an increased interest in herbal drug extracts, and this is due to several reasons, specifically, conventional medicine can be inefficient, abusive and or incorrect use of synthetic drugs results in side effects and a large percentage of the World's

population do not have access to conventional pharmacological treatment.

The exploration of ethnopharmacological treatments may be an important option in the treatment of parkinson's disease (Nagashayan et al., 2000) *Hemidesmus indicus* R. Br. is commonly known as Indian sarsaparilla belonging to Periplocaceae family (Shanti et al., 2010; Satheesh, 2008). It is a perennial climbing herb native of India and also found in Sri lanka, Pakistan, Iran and Bangladesh (Satheesh, 2008; Kurapati and Nishteswar, 2012). In Ayurveda it is one of the Rasayana plants, as it possesses anabolic effect. The plant also reported to be used in the treatment of syphilis, herpes, skin diseases, bronchitis, arthritis, gout, rheumatism, epilepsy, urinary diseases, chronic nervous diseases, loss of appetite, abdominal distention and intestinal gas (Kurapati and Nishteswar 2012; Rajan et al., 2011). The *Hemidesmus indicus* also possesses anticholinergic and CNS depressant effect which suggest the possible antiparkinsonian effect of the drug. In addition, root extract of the *Hemidesmus indicus* is known to possess significant anti-inflammatory, antioxidant and antipyretic activities (Shete and Bodhankar, 2010).

Haloperidol {4-(4-chlorophenyl)-1-(4-(4-flurophenyl)-4-oxobutyl)-4-piperidinol} is the widely used antipsychotic drug and it shares some structural similarity with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP is identified as the toxic agent present in heroin and responsible for neurodegenerative condition similar to parkinson's disease. MPTP is commonly used to induce parkinsonism in experimental animals. Haloperidol is metabolized in liver, it undergoes oxidation to the pyridinium metabolite, 4-(4-chlorophenyl)-1-(4-(4-fluorophenyl)-4-oxobutyl)-pyridinium (HPP^+) which shares some structural similarity and toxic actions with pyridinium metabolite of MPTP 1-methyl-4-phenylpyridine (MPP^+). This suggests that HPP^+ might produce neurological effects similar to MPTP (Tetsuhito et al., 2007). Therefore, in the present study haloperidol is used to induce parkinsonism in mice.

The aim of the present study is to explore the role of *Hemidesmus indicus* in the disorders of muscular disabilities (parkinsonism).

MATERIAL AND METHODS

Plant material & preparation of extract

The sample of *Hemidesmus indicus* (whole plant) was purchased from Munnalal Dawasaaz, Hyderabad, AP, India. The taxonomic evaluation was established by Prof. P. Jayaraman, Director, Institute of Herbal Botany, Plant Anatomy & Research Centre, Chennai, TN, India. The plant material was air dried under sunshine, cleaned & pulverized using a mechanical grinder. Fines were collected by sieving (40#). Fines were stored in air tight container at room temperature. 500 g of powdered drug in three batches (200, 200 & 100 g) were extracted successively in different solvents by continuous extraction process (Soxhlet apparatus) (Trease and Evans, 2009; Agarwal and Paridhavi, 2007). After completion of extraction it was filtered through whatman (no.1) filter paper and the solvent was removed by evaporation at room temperature. A dark brownish gummy mass of metahnolic extract of *Hemidesmus indicus* (21.5% w/w) was obtained, reconstituted with 1% carboxymethylcellulose (CMC), labeled and stored under refrigeration in screw cap bottles until further use.

Phytochemical analysis

The MEHI was obtained by successive solvent extraction and subjected to preliminary qualitative phytochemical analysis using standard methods for the identification of alkaloids, carbohydrates, glycosides, flavonoids, saponins, tannins, terpenes and phenolic compounds **(**Kokate, et al., 1994, Agarwal, et al., 2007, Shanthi, et al., 2010)

Animals

Swiss albino mice of either sex (20-30 g) were used. Animals were housed in well ventilated room (temperature 23 ± 2 ^oC, humidity 45-60% and 12 h light/dark cycle) at animal house, Department of Pharmacology, MESCO College of Pharmacy, Hyderabad, AP, India. All Animals were fed with standard pellet diet and had access to water *ad libitum.* The mice were randomly divided into five groups of six mice each. The experimental protocol were approved by the institutional animal ethics committee (1185/A/08/CPCSEA) and conducted in accordance with Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA) norms and the National Institute of Health Guidelines "Guide for the care and use of laboratory animals".

Drugs

Haloperidol (Mylan Laboratories, Hyderabad, AP, India) 2 mg/kg (i.p.) was used to induce parkinsonism/catalepsy (negative control) in animals (Bajaj and Vohora, 2000; Shimizu, 2004, Joshi et al., 2011) & Benzhexol (Provizer Pharma, Surat, Gujrat, India) 1 mg/kg (i.p.) was used as standard drug (positive control). Normal saline 1 ml/kg (i.p.) was used as normal control.

Gross behavioral studies

Two groups of six mice were used. Group I (normal control) given with 1% CMC (1 ml/kg), p.o. (Shimizu, 2004). The mice of group II were treated with MEHI (75 mg/kg, i.p.). The animals of both group were observed continuously for 1 hour for gross behavioral changes and then intermittently for the 6 hours followed by 24 hours.

Acute toxicity study

For acute toxicity study of MEHI, fixed dose (OCED Guideline No. 420) method of CPCSEA was adopted (Bhargava et al., 2009; Shirish, 2002). The acute toxicity of MEHI was determined in albino mice, maintained under standard conditions. The animals were fasted overnight prior to the experiment. The MEHI was administered in the dose of 1000 mg/kg (i.p.) to the group of animals containing six mice. The mortality, if any was observed after 24 hours.

Evaluation of antiparkinsonian activity

The 'Bar Test' was used to evaluate the antiparkinsonian activity of MEHI as it is the well established test to quantify the catalepsy in animals. This test determines the ability of the animal to respond to an imposed static posture (Mabrouk et al., 2010). Bar test is also known as catalepsy test and can be utilized to quantify akinesia, bradykinesia or dystonia which are the major disabilities associated with parkinsonism (Mabrouk et al., 2010, Bikash and Ajay, 2008). Haloperidol is widely used to induce parkinsonism like condition in the dose 0.5 to 2 mg/kg in mice (Joshi et al, 2011).

The mice were randomly divided into five groups of six (n=6) each. The treatment scheme is as follows:

- Group I animals were treated with vehicle control (saline 1 ml/kg) i.p. to observe the animal behavior.
- Group II animals were treated with haloperidol (2 mg/kg) i.p. to induce parkinsonism.
- Group III animals were treated with Benzhexol (1 mg/kg) i.p., one hour prior to the administration of haloperidol.
- Group IV animals were treated with low dose of MEHI (75 mg/kg) and haloperidol (2 mg/kg) i.p.
- Group V animal animals were treated with high dose of' MEHI (100 mg/kg) and haloperidol (2 mg/kg) i.p.
- Finally animals were observed for the onset and severity of parkinsonisonian response in all groups.

Initially Group I animals were treated with vehicle control (saline 1 ml/kg) i.p. Animals were placed on flat horizontal surface. Then both the forepaws of mice were placed on a wooden bar elevated (2 cm) above the ground to observe the animal behavior. The time in seconds that each paw spent on the block was recorded (Mabrouk et al., 2010, Mohanasundari et al., 2006). The group II animals were treated with haloperidol (2 mg/kg) i.p. to induce catalepsy and observed for response. Further, group III animals were treated with Benzhexol (1 mg/kg) i.p., 1 hour prior the administration of haloperidol and observed for the response. Followed by, the group IV animals were treated with low dose of MEHI (75 mg/kg) and haloperidol (2 mg/kg) i.p., observed for the response. The group V animals were treated with high dose of MEHI (100 mg/kg) (Rajgopal et al., 2010) along with haloperidol (2 mg/kg) i.p. and observed for the response. Finally groups were compared for the onset and severity of parkinsonism and antiparkinsonian potential respectively (Bajaj and Vohora, 2000; Mabrouk et al., 2010).

Scoring for the catalepsy

The cataleptic response was observed according to following scores:

0–Animal moved normally when placed on the table;

0.5–Animal moved only when touched or pushed;

0.5–Animal placed on table with front paws set alternatively on a 2 cm high wooden bar failed to correct the posture (120 sec set as cut off time). Time (in sec) taken to correct the posture was multiplied by the score for each paw;

1.0–Animal failed to correct the posture when front paws are placed on 4 cm high wooden bar. Time (in sec) taken to correct the posture was multiplied by the score for each paw;

Catalepsy score was calculated according to following formula:

Total score = 0.5 + (0.5 × Time in sec of front right paw on 2 cm high wooden bar) + (0.5 × Time in sec of front left paw on 2 cm high wooden bar) + (1× Time in sec of front right paw on 4 cm high wooden bar) + (1× Time in sec of front left paw on 4 cm high wooden bar) (Joshi et al., 2011, Bikash, and Ajay, 2008, Kulkarni, 2004).

Note: Mouse move only when touched or pushed then score 0.5. Then mouse placed on table with front right paw on 2 cm high wooden bar, fails to correct the posture in specific time (in sec) suppose 100 sec, the score 0.5 multiplied by the time taken to correct the posture, the score become 0.5 × 100 = 50. Similarly suppose left paw, taking time 90 sec to correct the posture, score become 0.5 × 90 = 45. In case of right paw placed on 4 cm high bar, taking time of 60 sec to correct the posture, the score will be 1 × 60 = 60. Similarly with left paw, taking 80 sec to correct the posture, score will be 1 × 80 = 80.

Statistics

The data were expressed as the mean ± standard error of mean (SEM). Data were analyzed by one-way ANOVA followed by Student's'*t*'-test & the level of statistical significance adopted was $P < 0.05$.

RESULTS

Phytochemical analysis

The methanolic extract obtained after successive solvent extraction was subjected to preliminary qualitative phytochemical investigation. This type of analysis helps in determining the presence of main chemical constituents of the herbs which are responsible for the specific biological or pharmacological activity. Results of these tests are presented in Table 1.

Table 1. Qualitative chemical analysis of MEHI.

Sr. No.	Test/Reagent used	Observation
1.	Alkaloids	-
2.	Carbohydrates	+
3.	Glycosides	+
4.	Flavonoids	+
5.	Saponins	-
6.	Tannins	+
7.	Terpenoids	-
8.	Phenolic compounds	+
9.	Proteins and Lignin's	+

+ = Present - = Absent

The phytochemical investigation of MEHI indicated the presence of carbohydrates, glycosides, flavonoids, tannins and phenolic compounds while alkaloids, saponins, lignins, proteins while terpenoids were found to be absent.

Gross behavioral studies

Two groups of six mice were used, group I and group II showed the same response. Respiration, sense of touch & sound were found to be present in all mice while writhing, tremor, convulsions, salivation, diarrhea and mortality were found to be absent in all mice (Table- 2).

Table 2. Gross behavioral studies of MEHI on mice.

Gross behavior	Observation							
	Up to 1h	2h	3h	4h	41/2h	6h	12h	24h
Respiration	+	+	+	+	+	+	+	+
Writhing	-	-	-	-	-	-	-	-
Tremor	-	-	-	-	-	-	-	-
Convulsions	-	-	-	-	-	-	-	-
Salivation	-	-	-	-	-	-	-	-
Hind limb paralysis	-	-	-	-	-	-	-	-
Diarrhea	-	-	-	-	-	-	-	-
Sense of touch & sound	+	+	+	+	+	+	+	+
Mortality	-	-	-	-	-	-	-	-

+ = Present, - = Absent

Acute toxicity study

It was observed that MEHI was safe to use in albino mice & showed no mortality on intra peritoneal administration of 1000 mg/kg dose. MEHI had no unwanted effects on the normal behavior of the test animals. The dose of 75 mg/kg body weight & 100 mg/kg body weight was selected for the experiment as maximal dose.

Antiparkinsonian activity

In the present study, haloperidol (2 mg/kg i.p.) was administered to mice to induce catalepsy, both the forepaws of animals were placed on a wooden bar and cataleptic response (score) was measured according to the mentioned formula. These cataleptic scores were recorded against time in seconds and the following observations were drawn:

- Onset of overall catalepsy was the time at which animal started showing catalepsy
- Duration of overall catalepsy was the total duration of catalepsy.
- Onset of maximum catalepsy was the time at which animals initiated to show maximum score of catalepsy (335).
- Duration of maximum catalepsy was the duration maximum score of catalepsy i.e. 335.

Table 3 shows the effect of MEHI on induction and duration of catalepsy as observed in change in catalepsy score with respect to time in haloperidol-induced catalepsy. Group I (normal control) normal saline treated animals did not displayed catalepsy as they scored less than 0.5 on the bar at each time point. Group II (negative control) haloperidol treated mice showed strong cataleptic state. While group III (positive control) benzhexol treated animals showed decrease in onset and duration of catalepsy as compare to group II animals. Pretreatment with MEHI 75 & 100 mg/kg (test drug; group IV & V) reduced the catalepsy score i.e. delayed onset and shortened duration of catalepsy in haloperidol treated mice significantly ($P < 0.05$ and $P < 0.01$). MEHI at dose levels of 75 & 100 mg/kg produced protective effect against haloperidol-induced catalepsy in mice (Table 3).

DISCUSSION

In the present study an attempt was made to evaluate the antiparkinsonian activity of MEHI against parkinsonism induced by haloperidol in *Swiss* albino mice. After performing the gross behavioral studies antiparkinsonian activity was evaluated. Haloperidol produced strong cataleptic response in animals. Pretreatment with MEHI (75 & 100 mg/kg i.p.) reduced the catalepsy score in haloperidol treated mice. We found that the effective dose of MEHI

Table 3. Effect of *Hemidesmus indicus* extract on haloperidol-induced parkinsonism (catalepsy).

S. N.	Groups	Drug dose (mg/kg)	Overall cataleptic posture (seconds)		Maximum cataleptic posture (seconds)	
			Onset	Duration	Onset	Duration
1	**Group –I (Normal control)** (Normal saline; 0.9% NaCl)	1 ml/kg (0.9% NaCl)	No Cataleptic response	No Cataleptic response	No Cataleptic response	No Cataleptic response
2	**Group–II (Negative control)** (Haloperidol)	2 mg/kg	85 ± 2.12	217 ± 6.00	97 ± 2.56	152 ± 14.24
3	**Group–III (Positive control)** (Benzhexol + Haloperidol)	1 mg/kg + 2 mg/kg	115 ± 1.10*	155 ± 7.00**	145 ± 2.6**	140.33 ± 9.24**
4	**Group–IV (Test-low dose)** (MEHI + Haloperidol)	75 mg/kg + 2 mg/kg	110 ± 2.14*	163 ± 05**	141 ± 3.4	139.28 ± 6.09*
5	**Group–V (Test-high dose)** (MEHI + Haloperidol)	100 mg/kg + 2 mg/kg	119 ± 2.11**	153 ± 2.3**	150 ± 3*	133.21 ± 4.11**

MEHI: Methanolic extract of *Hemidesmus indicus*

Values are mean ± SEM (n=6); *P <0.05, **P < 0.01. All the test groups compared with negative control group.

is much less as compared to other herbal drugs (e.g. ocium sanctum) to produce antiparkinsonian effect in rodents. This evaluation of anticataleptic activity of drug is the classical model for evaluating the antiparkinsonian effect of the drug (Joshi et al., 2011). As tremors, muscular rigidity & abnormalities of posture & gait are the major symptoms associated with parkinsonism. Despite the widespread use of *Hemidesmus indicus* for treating various ailments, there is hardly any scientific evaluation of its CNS activity in general & specifically antiparkinsonian activity. Earlier reports on chemical constituents of plants & their correlation with CNS activity suggests that plant containing flavonoids, tannins, saponins & phenols may possess antiparkinsonian effect (Mohanasundari et al., 2006, Sokindra et al., 2008, Shikha et al., 2010). In the present study the quoted constituents found to be present in *Hemidesmus indicus*.

CONCLUSION

In conclusion, it is suggested that the *Hemidesmus indicus* has antiparkinsonian activity against haloperidol-induced parkinsonism in albino mice. Phenols and tannins may be the responsible constituent for its antiparkinsonian effect. Our efforts are in progress to isolate the compound responsible for the antiparkinsonian activity of the *Hemidesmus indicus* and to evaluate the biochemical basis of its action. In addition more studies using different *in vivo* and *in vitro* models including clinical trials are needed to establish its effect as antiparkinsonian.

CONFLICT OF INTEREST

None declared.

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