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POTENTIAL ANTIPARKINSONIAN AND ANTIDEPRESSANT EFFECTS OF METHANOLIC EXTRACT OF *SWERTIA CHIRATA* AND *HEMIDESMUS INDICUS* IN WISTAR RATS

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ABSTRACT
Parkinsonism is neurodegenerative disorder that causes combination of the movement abnormalities such as tremor, slow movement, impaired speech and muscle stiffness mainly due to the loss of dopamine containing neurons. Use of herbal drugs is an important initiative in the treatment of chronic diseases. In the present study we evaluated antiparkinsonian activity of two popular herbal drugs *Swertia chirata* and *Hemidesmus indicus* against haloperidol-induced parkinsonism (Catalepsy) in wistar rats. MESC and MEHI were prepared by successive solvent extraction (Soxhlet apparatus) & two doses 75 & 100 mg/kg i.p. of MESC and MEHI were evaluated for antiparkinsonian activity. Benzhexol was used as reference standard drug. Normal saline was used as a control. Pretreatment of test animals with *Swertia chirata* and *Hemidesmus indicus* (75 & 100 mg/kg i.p.) reduced the catalepsy score in haloperidol treated rats. Both (75 & 100 mg/kg i.p) doses of *Swertia chirata* and *Hemidesmus indicus* were protective against haloperidol-induced catalepsy ($P < 0.05$ and $P < 0.01$). These herbal drugs also showed significant antidepressant effect. Thus the present study suggests that the *Swertia chirata* and *Hemidesmus indicus* has antiparkinsonian and antidepressant activity. Flavonoids, Phenols and Terpenes may be the responsible leads for our present antiparkinsonian activity. Further in depth studies are needed to explore & establish the role of these chemical constituents.

KEYWORDS: Methanolic Extract of *Swertia Chirata* (MESC) and Hemidesmus indicus (MEHI), Muscular Rigidity, Catalepsy, Haloperidol.

INTRODUCTION
Parkinsonism is a progressive, age-related second most common neurodegenerative disorder, affecting 1.4% of the global population above the age of 55 years.[1,2] An estimated 5 million people worldwide have Parkinson's disease, with one million people each in the US and Europe.[1,3] It is estimated that about 2% of people over 65 years of age are sufferers of Parkinson's disease.[2] Amid the aging of the population 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.[1,3] Parkinson's disease was first described by James Parkinson in 1817. He described this neurological disorder as shaking palsy and inscribes a monograph entitled "An essay on the shaking palsy".[1,4] Parkinsonism is progressively affects and 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).[5] In addition disturbances of equilibrium and autonomic functions frequently occur. These symptoms are due to low levels of dopamine in the fore brain area.[1,3,6] 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.[6] 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 moreover they are expensive.[7,8]

The use of herbal drugs 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. This is due to

several reasons, specifically, conventional medicine can be inefficient, abusive and or incorrect use of synthetic drugs results in side effects. Moreover a large percentage of the World's population do not have access to conventional pharmacological treatment.[9] The exploration of ethnopharmacological treatments may be an important alternative in the treatment of parkinson's disease.[10] *Hemidesmus indicus* R. Br. commonly known as Indian sarsaparilla belonging to family Periplocaceae.[11,12] It is a perennial climbing herb native of asian countries viz. India, Sri lanka, Pakistan, Iran and Bangladesh [12,13] 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 flatulence.[13.14] The *Hemidesmus indicus* also possesses anticholinergic, antioxidant, antipyretic, anti-inflammatory and CNS depressant effect which suggest the potential antiparkinsonian effect of the drug.[15] Swertia chirata Buch-Ham (Fam. Gentianaceae) is well known for its medicinal properties. The chemical constituents like amarogentin, swerchirin, swertiamarin and other active principles of the herb possesses the bitterness, antihelmintic, hypoglycemic and antipyretic properties. Herbal formulations such as Ayush-64, Diabecon, Mensturyl syrup, Melicon V ointment & Mahasudarshan churna contain Swertia chirata Powder & its extract in different amounts for its antipyretic, hypoglycemic, antifungal and antibacterial properties.[16,17] The Xanthone derivatives of the herb like mangostin, isomangostin and mangostin triacetate-3 are identified to possess significant anti-inflammatory and CNS stimulant effect.[18,19]

Haloperidol {4-(4-chlorophenyl)-1-(4-(4-flurophenyl)-4-oxobutyl)-4-piperidinol} is the widely used antipsychotic drug which 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 which is 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.[19,20] Hence, in the present study haloperidol is used to induce parkinsonism in rats.

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

MATERIAL AND METHODS

Plant material & preparation of extract

The samples of *Swertia chirata* and *Hemidesmus indicus* (whole plant) were purchased from Munnalal Dawasaaz, Hyderabad, AP, India. The taxonomic evaluation of plants done by Prof. P. Jayaraman, Director, Institute of Herbal Botany, Plant Anatomy & Research Centre, Chennai, TN, India. The plant materials were air dried under sunshine, cleaned & pulverized using a mechanical grinder. Fines were collected by sieving (40#) and stored in air tight container at room temperature. 500 g of powdered drugs in three batches (200, 200 & 100 g) were extracted successively in different solvents by continuous extraction process (Soxhlet apparatus).[21,22] After extraction, 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 *Swertia chirata* (18.5% w/w) and *Hemidesmus indicus* (21.5% w/w) were obtained. The extract were reconstituted with 1% carboxymethylcellulose (CMC), labeled and stored under refrigeration in screw cap bottles until further use.

Phytochemical analysis: The MEHI and MESC obtained by successive solvent extraction were subjected to preliminary qualitative phytochemical analysis. Standard methods were used for the identification of alkaloids, carbohydrates, glycosides, flavonoids, saponins, tannins, terpenes and phenolic compounds.[11,22,23]

Animals: Adult Wistar rats of either sex (170-200 g) were used. Animals were housed in well ventilated room (temperature 23±2^{o}C, humidity 45-60% and 12 h light/dark cycle) at animal house, Department of Pharmacology, MESCO College of Pharmacy, Hyderabad, AP, India. All Animals had access to water *ad libitum* and were fed with standard pellet diet. The animals were randomly divided and allocated to treatment groups. 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: Benzhexol (Provizer Pharma, Surat, Gujrat, India) 2 mg/kg (i.p.) was used as standard drug (positive control). Haloperidol (Mylan Laboratories, Hyderabad, AP, India) 4 mg/kg (i.p.) was used to induce parkinsonism/catalepsy (negative control) in animals & Normal saline 3 ml/kg (i.p.) was used as normal control. For antidepressant activity Imipramine (Sigma-Aldrich laboratories ltd) 30 mg/kg is used as standard drug.[16,23,24]

Gross behavioral studies: Three groups of six rats were made. Group I (normal control) given with 1% CMC (1 ml/kg), p.o. The rats of group II were treated with MESC

(100 mg/kg, i.p.). Group III animals were treated with MEHI (100 mg/kg, i.p.) The animals of all the groups were observed continuously for 1 hour for gross behavioral changes and then intermittently for the 6 hours followed by 24 hours.[24]

Acute toxicity study

For acute toxicity study of MESC and MEHI, fixed dose (OCED Guideline No. 420) method of CPCSEA was adopted. The acute toxicity was determined in wistar rats, maintained under standard conditions. The animals were fasted overnight prior to the experiment. The drugs were administered in the dose of 1000 mg/kg (i.p.) to the group of animals containing six rats. The mortality, if any was observed after 24 hours.[17, 25]

Evaluation of antiparkinsonian activity

The "Bar Test" was used to evaluate the antiparkinsonian activity of MESC and 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.[26] Bar test is also known as catalepsy test and can be employed to quantify akinesia, bradykinesia or dystonia which are the major disabilities associated with parkinsonism. Haloperidol is widely used to induce parkinsonism like condition in the dose 0.5 to 4 mg/kg in rats.[16,26, 27]

The rats were randomly divided into two main groups each for MESC and MEHI. Further five groups of six animals each (n=6) were made. The treatment scheme followed for each subgroup is as follows

- Group I animals were treated with vehicle control (saline 3 ml/kg) i.p. to observe the animal behavior.
- Group II animals were treated with haloperidol (4 mg/kg) i.p. to induce parkinsonism.
- Group III animals were treated with Benzhexol (2 mg/kg) i.p., one hour prior to the administration of haloperidol.

The above scheme is same for both groups of MESC and MEHI.

- Group IV animals were treated with low dose of MESC (75 mg/kg) and haloperidol (4 mg/kg) i.p.
- Group V animal animals were treated with high dose of' MESC (100 mg/kg) and haloperidol (4 mg/kg) i.p.

And for MEHI-

- Group IV animals were treated with low dose of MEHI (75 mg/kg) and haloperidol (4 mg/kg) i.p.
- Group V animal animals were treated with high dose of' MEHI (100 mg/kg) and haloperidol (4 mg/kg) i.p

The animals were observed for the onset and severity of parkinsonisonian response in all groups. Animals were placed on flat horizontal surface. Afterwards both the forepaws of rats were placed on a wooden bar elevated (9 cm) above the ground to observe the animal behavior. The time in seconds that each paw spent on the block was recorded. Finally all the groups were compared for the onset and severity of parkinsonism and antiparkinsonian potential respectively.[23,26, 28]

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 4 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 9 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 4 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 9 cm high wooden bar).[16,27.29]

Note: Rat move only when touched or pushed then score 0.5. Then rat placed on table with front right paw on 4 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 9 cm high bar, taking time of 60 sec to correct the posture, the score will be 1 × 60 = 60. Likewise with left paw, taking 80 sec to correct the posture, score will be 1 × 80 = 80.

Antidepressant activity

Forced swimming test (FST)

Behavioral despair is a well accepted model to test antidepressant activity in experimental animals. The procedure described by Porsalt et al(1978) was adopted for this test.[30] Rats of either sex were divided in six different groups and were individually placed to swim in an open cylindrical container (25 cm high and 10 cm wide), containing 19 cm of (25±1 °C) water. Group I animals were treated with vehicle control (saline 3 ml/kg). The other four groups received low and high doses of MESC and MEHI (75 and 100 mg/kg). The sixth group received standard drug Imipramine (30 mg/kg). Before the actual test, trial swimming session were performed. After 24 hrs the animals were exposed to test for 10-min period. During the last 6 min the total duration of immobility was recorded with the help of stop watch. The animals were judged to be immobile whenever it stopped struggling and remained floating motionless in the water, keeping the nose just above the water level. Reduction in the duration of immobility has been recorded.[31, 32,33]

Tail suspension test (TST)

All the animals of either sex were divided and treated same as described for FST. The method described by Steru *et al*., (1985) has been adopted in our study. The animals were suspended by tail on a rod set at 75 cm height using adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was noted during a 6 min period. The animals were considered immobile when they did not show any movement and hung inactively.[32,33]

BIOCHEMICAL INVESTIGATION

Lipid Peroxidation

The quantification of thiobarbituric acid reactive substances (TBARS) is an index of lipid peroxidation in rat brain tissue. TBARS and Malondialdehyde (MDA) were quantified by their reactivity with Thiobarbituric acid (TBA) in acidic conditions. 500 mg of rat brain was homogenized with normal saline and centrifuged at 4000 rpm for 10 minutes. The supernatant was collected and used for estimation of lipid peroxidation. 1ml of supernatant was added to 2 ml of reaction mixture (mixture of equal parts of the reagent 1, 2 and 3). The reaction solutions were kept in water bath for 15 minutes at 80˚C, cooled and centrifuged at 1500 rpm for 10 minutes. The reaction gives pink colour which was measured at 535 nm against a reagent blank. 1, 1, 3, 3 tetra methoxy propane was used as external standard. The amount of TBARS was expressed as nmoles/mg for haemolysate or nmoles/mL for plasma.[34, 35,36,37]

ESTIMATION OF ANTIOXIDANT ENZYMES

The enzymatic antioxidants analyzed were catalase and superoxide dismutase.

Estimation of Catalase (CAT)

The UV light absorption of hydrogen peroxide can be measured between 230 – 250 nm. The absorption decreases with time, on decomposition of hydrogen peroxide by catalase. The enzyme activity can be measured by this decrease in absorption. 0.1 ml of supernatant was added to cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7.0). Reaction was started by the addition of 1.0 ml of freshly prepared 30 mM hydrogen peroxide. The rate of decomposition of H2O2 was measured spectrophotometrically from changes in absorbance at 240 nm. Activity of catalase was expressed as units/mg protein. A unit has been defined as the velocity constant per second.[38,39,40]

Estimation of superoxide dismutase (sod)

The superoxide dismutase causes the photochemical reduction of riboflavin and catalyses the inhibition of Nitro blue tetrazolium (NBT) reduction, the extent of which can be assayed spectrophotometrically at 600nm. In 5ml of potassium phosphate buffer 0.5g of rat brain tissue was homogenized. After centrifugation at 2000 rpm for 10 minutes the supernatants were collected and used for the assay. 3.0 ml of incubation medium consist of, 50 mM potassium phosphate buffer (pH 7.8), 45 µM methionine, 5.3 mM riboflavin, 84 µM NBT and 20 µM potassium cyanide. The amount of homogenate added to this medium was kept below one unit of enzyme to ensure significant accuracy. The tubes were placed in an aluminium foil-lined box, equipped with 15W fluorescent lamp maintained at 25°C. The reduced NBT was measured spectrophoto-metrically at 600nm. The maximum reduction was noted in the absence of the enzyme. Enzyme activity in one unit was defined as the amount of enzyme giving a 50% inhibition of the reduction of NBT. The values were expressed as units/mg protein.[38,39,40]

Assay of Dopamine

Principle: The assay of dopamine (3-hydroxytyramine) was carried out by trihydroxyindole method. The dopamine is easily oxidized to a red indole derivative and in absence of oxygen undergoes an intra molecular rearrangement to form 5,6-dihydroxyindole. The second step is accelerated by the addition of alkali. The optimum pH for oxidation was 6.5.

As an antioxidant sulfite is preferred over ascorbate as it interferes with fluorescence. After rearrangement in alkali, pH should be adjusted to 5.3 by addition of acetic acid. After the final pH adjustment the spontaneous rise in fluorescence intensity is slow.

Procedure

To 1ml of above acid extract 0.5 ml, 0.1M PO_4 buffer of pH 6.5 was added followed by addition of 0.05 ml of 0.02 N of Iodine solutions. After 5 minutes 0.5 ml of alkaline sulfite solution was added to test and 0.5 ml of 2.5 N NaOH was added to blank. Again after 5 minutes 0.6ml of 2.5 N acetic acid was added to both test and blank and these were then irradiated under UV (240 nm) for 20 minutes. After irradiation, 0.05 ml water was added to the test and 0.05ml alkaline sulfite solution was added to blank. The fluorescence was read in a spectro-fluorimeter with activation and fluorescence peaks being 335 nm and 378 nm respectively. Readings for blanks were corrected and values of unknowns were obtained by extrapolation from the standard curve.[41,42]

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 and/or Dunnett's 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. The analysis may 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 (MESC) and Table 2 (MEHI). The phytochemical investigation of MESC

indicated the presence of alkaloids, carbohydrates, glycosides, flavonoids, Terpenoids and phenolic compounds while saponins, lignin's and tannins were absent. The phytochemical investigation of MEHI showed the presence of carbohydrates, glycosides, flavonoids, tannins and phenolic compounds whereas alkaloids, saponins, lignin's, proteins and terpenoids were absent.

Gross behavioral studies

Three groups of six rats were used, all the groups showed the same response. Respiration, sense of touch & sound were found to be present in all rats while writhing, tremor, convulsions, salivation, diarrhea and mortality were found to be absent in all rats (Table- 3).

Acute toxicity study

It has been observed that MESC and MEHI were safe to use in wistar rats & showed no mortality on intra peritoneal administration of 1000 mg/kg dose. These herbal drugs 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 were selected for the experiment as maximal dose.

Antiparkinsonian activity

In the present study, rats were treated with haloperidol (4 mg/kg i.p.) 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

- The time at which animal started showing catalepsy was the onset of overall 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 4 (MESC) and 5 (MEHI) show the effect of study drugs on induction and duration of catalepsy. Effects were observed as change in catalepsy score with respect to time in haloperidol-induced catalepsy. Data of both the groups (MESC and MEHI) indicates Group I (normal control) normal saline treated animals have not shown cataleptic response as they scored less than 0.5 on the bar at each time point. Group II (negative control) haloperidol treated rats 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 MESC and MEHI 75 & 100 mg/kg (test drugs; group IV & V) reduced the catalepsy score i.e. delayed onset and shortened duration of catalepsy in haloperidol treated rats significantly ($P < 0.05$ and $P < 0.01$). MESC and MEHI at dose levels of 75 & 100 mg/kg produced protective effect against haloperidol-induced catalepsy in rats (Table 4 and Table 5).

Antidepressant activity

The present results of both FST and TST suggests that MESC and MEHI at dose levels of 75 & 100 mg/kg body weight reduced the immobility time ($P<0.01$) as compared to the immobility time of control. The MEHI at dose of 100 mg/kg shown the best results when it was compared with control and standard. The decrease in the immobility time in MESC and MEHI treated animals was fairly close to standard. The results are summarized in Table- 7 and 8. These results show that the MESC and MEHI have antidepressant potentials.

Biochemical investigations

TBARS Activity

The TBARS levels were found to be significantly improved in the brain tissue of the animals treated with haloperidol (Table 9). MESC and MEHI have minimized the level of TBARS activity towards normal level dose dependently.

SOD Activity

In the animals pretreated with MESC and MEHI the levels of SOD were significantly increased as compared to haloperidol treated rats (Table 9).

CATALASE Activity

MESC and MEHI significantly raised the level of catalase towards normal level and the results were comparable to standard (Table 9).

Assay of Dopamine

Dopamine concentration in rat brain was determined in various groups. The present study shows that haloperidol reduced the dopamine concentration while MESC and MEHI restored the dopamine level towards normal level (Table 9).

Table 1: Qualitative chemical examination of methanolic extract of *Swertia chirata* (MESC)

Sr. No	Test/Reagent used	Observation
1	**Alkaloids**	+
2	**Carbohydrates**	+
3	**Glycosides**	+
4	**Flavonoids**	+
5	**Saponins**	-
6	**Tannins**	-
7	**Terpenes**	+
8	**Phenolic compounds**	+

Table 2: Qualitative chemical analysis of methanolic extract of Hemidesmus indicus (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

Table 3: Gross behavioral studies

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

+ = Present, - = Absent

Table 4: Acute toxicity study

Drug	Dose (mg/kg body weight)	No. of Animals Used	No. of Death	Percentage Death
MESC	**1000**	**06**	**0**	**0**
MEHI	**1000**	**06**	**0**	**0**

MESC-Methanolic extract of *Swertia chirata.* MEHI- Methanolic extract of *Hemidesmus indicus*

Table 5: Effect of *Swertia chirata* extract on haloperidol-induced Parkinsonism (catalepsy)

Groups	Drug Dose (mg/kg)	Overall Cataleptic(Abnormal) posture (Seconds)		Maximum cataleptic (Abnormal) posture (Seconds)	
		Onset	Duration	Onset	Duration
Group – I (Control) Normal saline 0.9%NaCl)	**1 ml/kg (0.9% NaCl)**	**No Cataleptic response**	**No Cataleptic response**	**No Cataleptic response**	**No Cataleptic response**
Group-II (Neurotoxic) Haloperidol	4 mg/kg	80±2.12	212±6.00	92±2.30	150± 12.14
Group – III (STD) Benzhexol + haloperidol	2 mg/kg + 4 mg/kg	113±1	151±7.11**	144±2.5**	134.33±9.2**
Group – IV(Test Low dose) MESC + Haloperidol	75 mg/kg + 4 mg/kg	112±2.*	162.03±05**	143±2.3	137.28±5.10*
Group – V(Test High dose) MESC + Haloperidol	100 mg/kg + 4 mg/kg	120±2.00**	152±2.13**	149±2.7*	131.2±3.12**

MESC: Methanolic extract of *Swertia chirata.*

Values are mean ±SEM (n=6); **P* <0.05, ***P* < 0.01.

All the test groups compared with negative control group.

Table-6 Effect of *Hemidesmus indicus* extract on haloperidol-induced Parkinsonism (catalepsy)

Groups	Drug Dose (mg/ kg)	Overall Cataleptic(Abnormal) posture (Seconds)		Maximum cataleptic (Abnormal) posture (Seconds)	
		Onset	Duration	Onset	Duration
Group – I (Control) Normal saline 0.9%NaCl)	**1 ml/kg (0.9% NaCl)**	**No Cataleptic response**	**No Cataleptic response**	**No Cataleptic response**	**No Cataleptic response**
Group-II (Neurotoxic) Haloperidol	4 mg/kg	85±2.12	217±6.00	97 ± 2.56	152 ±14.24
Group – III (STD) Benzhexol + haloperidol	2 mg/kg + 4 mg/kg	115±1.10*	155±7.00**	145 ±2.6**	140.33±9.24**
Group – IV(Test Low dose) MEHI + Haloperidol	75 mg/kg + 4 mg/kg	110±2.14*	163±05**	141 ± 3.4	139.28 ± 6.09*
Group – V(Test High dose) MEHI + Haloperidol	100 mg/kg + 4 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.

Table 7- Effect of Methanolic extract of *Swertia chirata* (MESC) and *Hemidesmus indicus* (MEHI) on duration of immobility time in tail suspension test (TST)

Groups	Drug Treatment	Dose mg/kg	Duration of Immobility (Seconds)
Group – I	Normal saline 0.9%NaCl	3ml/kg	187±3.21
Group-II	Std drug Treament Imipramine	30	133±3.91**
Group –III	Low dose of MESC	75	147±5.93*
Group – IV	High dose of MESC	100	143±4.43**
Group – V	Low dose of MEHI	75	142±3.51*
Group – VI	High dose of MEHI	100	137±4.31**

Values are mean ±S.E.M. (n=6); **P* <0.05, ***P* < 0.01.
Data analysis was performed using Dunnett's test.
All the test groups compared with normal control group

Table 8- Effect of Methanolic extract of *Swertia chirata* (MESC) and *Hemidesmus indicus* (MEHI) on duration of immobility time in forced swim test (FST)

Groups	Drug Treatment	Dose mg/kg	Duration of Immobility (Seconds)
Group – I	Normal saline 0.9%NaCl	3ml/kg	182
Group-II	Std drug Treament Imipramine	30	123±2.21**
Group –III	Low dose of MESC	75	141±3.33*
Group – IV	High dose of MESC	100	139
Group – V	Low dose of MEHI	75	140±2.1*
Group – VI	High dose of MEHI	100	135±2.21**

Values are mean ±S.E.M. (n=6); *P <0.05, **P < 0.01.
Data analysis was performed using Dunnett's test.
All the test groups compared with normal control group

Table 9- Effect of MESC and MEHI on lipid peroxidation and antioxidant enzymes.

Groups	Drug Treatment	TBARS (nmoles MDA/mg protien)	SOD (Units/mg proteins)	CAT (NmoleH2O2/mg protein)
Group – I	Normal saline 0.9%NaCl	28.29±0.07	35.3±0.15	38.27±0.58
Group-II	Neurotoxic Haloperidol	55.77±0.12***	23.1±0.13***	20.8±1.49***
Group –III	Low dose of MESC	34.11±0.15***###	33.1±0.15***###	32.41±0.39*###
Group – IV	High dose of MESC	43.13±0.29***###	27.89±0.11***###	26.2±1.33***#
Group – V	Low dose of MEHI	39.44±0.19***###	29.93±0.13***###	28.8±1.33***##
Group – VI	High dose of MEHI	40.34±0.11***###	28.99±0.12***###	27.9±0.13***###

The values are expressed as Mean ± SEM (n=6). The data were analyzed by using Oneway ANOVA followed by dunnet's test.
***P<0.001,*P<0.05 as compared to group I control; ###P<0.001, ##P<0.01, #P<0.05 as compared to group II Negative control.

Table 10- Effect of MESC and MEHI on concentration of Dopamine(nM) in rat brain tissue.

Sr. No.	Sample Name	Concentration of Dopamine(nM)
1	Control(Blank)	0.00
2	Normal Control (N.Saline)	8.10±0.03
3	Neurotoxic (Haloperidol)	2.95±0.01***
4	Standard (Benzhexol + Haloperidol)	6.10±0.10***###
5	Low dose of MESC	4.60±0.08***###
6	High dose of MESC	5.03±0.03***###
7	Low dose of MEHI	4.80±0.01***###
8	High dose of MEHI	5.11±0.07***###

The values are expressed as Mean ± SEM. The data were analyzed by using One way ANOVA followed by dunnet's test.
***P<0.001 as compared to group I control; ###P<0.001 as compared to group II Neurotoxic.

DISCUSSION AND CONCLUSION

In the present study an attempt was made to evaluate the antiparkinsonian activity of Methanolic Extract of *Swertia chirata* and *Hemidesmus indicus* against Parkinsonism induced by haloperidol in wistar rats. After performing the gross behavioral studies antiparkinsonian activity was evaluated. Haloperidol produced strong cataleptic response in animals. Pretreatment with Methanolic Extract of *Swertia chirata and Hemidesmus indicus* reduced the catalepsy score in haloperidol treated rats. Evaluation of anticataleptic activity of drug is the classical model for evaluating the antiparkinsonian effect of the drug. As tremors, muscular rigidity & abnormalities of posture & gait are the major symptoms associated with Parkinsonism.[26,28] Despite the widespread use of *Swertia chirata* and *Hemidesmus indicus* for treating various ailments, there is hardly any scientific evaluation of CNS activities in general & specifically antiparkinsonian activity.[16] Earlier reports on chemical constituents of plants & their correlation with CNS activity suggests that plant containing Flavonoids, Tannins, Saponins, Terpenoids, Xanthones & Phenols may possess antiparkinsonian effect.[10] Phytochemical evaluation revealed that *Swertia chirata* and *Hemidesmus indicus* contains these compounds. These drugs also showed antidepressant effects which may be helpful for potentiating antiparkinsonian activity.

Thus, in conclusion, the findings of the present study suggest the *Swertia chirata* and *Hemidesmus indicus* has potential antidepressant and antiparkinsonian activity against haloperidol-induced Parkinsonism in wistar rats. Flavonoids, Phenols and Terpenoids may be the responsible constituent for antiparkinsonian effect. Further studies using different in vivo and in vitro models including clinical trials are needed to explore the Antiparkinsonian effect of these drugs.

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