The Effect of *Biophytum sensitivum* Extract Against the Behavioral Changes Induced by 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP) in mice

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**Abstract**

Medicinal plant *Biophytum sensitivum* was studied for anti-parkinsonian effects in MPTP induced Parkinsonism mice. This herb was selected after an extensive survey of the folk literature and advice from the practicing physicians of Siddha systems of medicine. This plant is used in indigenous medicine against various health conditions. The objective of the present work is to study the synergistic neuroprotective effects of *B. sensitivum* alcoholic extract in MPTP induced Parkinsonian mice. Behavioral studies were performed by the actophotometer, elevated plus maze, rotarod, hole board, step down and step through tests. In the PD mouse grooming, stride length, movement, rearing were found to be decreased compared to the control. The result obtained in the present study reveals that different doses of *B. sensitivum* increased memory retention and retrieval significantly. The *B. sensitivum* extract is more in antioxidants which may produce a considerable improvement in the enzyme activity and reduce oxidative stress, which plays a significant role in the toxicity reduction of MPTP.

**Key words:** Behaviour, MPTP, *Biophytum sensitivum*, Parkinson’s disease

**INTRODUCTION**

Parkinson’s disease (PD or simply idiopathic Parkinsonism), is one of the most widespread progressive neurodegenerative disease found in the aging population. There are more than 120 medicinal plants that are being used for the treatment of Central Nervous System (CNS) disorders in Asian countries. In recent times plant based medicines are gaining priority in developed pharmaceutical market because of availability, safety and no major regulatory controls. This study was made towards search for effective and safe alternatives from herbs to cure PD. There is evidence that plant based antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in spices, herbs, and medicinal plants. In recent drug development, a number of natural active constituents have been identified that could potentially use in prevention and treatment of diseases. One plant that has been used in CNS illnesses is *Biophytum sensitivum*. It is commonly called as life plant or sensitive plant and belongs to the family Oxalidaceae. It is one of the auspicious herbs that constitute the group "Dasapushpam", an Ayurvedic formulation. *B. sensitivum* is used as indigenous medicine to cure diversity of diseases. Strategies employing antioxidant and neuroprotective from natural sources can be a high-quality approach in improving the healing of Parkinson’s disease. So efforts have been made in the present study to investigate the effects of *B. sensitivum* on animal models of Parkinson’s disease.

**EXPERIMENTAL**

**Chemicals and Reagents**

MPTP hydrochloride was purchased from Sigma Chemical Co. All other chemicals used were of analytical grade. Stock solutions of all chemicals were prepared in distilled water and the dilutions were made fresh on the day of the experiment.

**Plant Extract**

The medicinal plant *B. sensitivum* was collected from Tirunelveli District, Tamil Nadu, India. Mature and healthy plants were collected naturally from diverse locations after the rainy season (February, March and April). The specimens were identified referring to the Flora of Presidency of Madras and Flora of Tamil Nadu Carnatic. The specimens were shade-dried at room temperature (18-20°C) for a period of 3 weeks to 8 weeks. The completely dried materials were prepared in to coarse powder by mechanical grinder and the powder was passed through a 40-mesh sieve, to get a uniform particle size and then used for extraction purpose. A weighed quantity of powder was subjected to continuous hot percolation in soxhlet apparatus with ethanol at 65-70°C. The extracts were evaporated under reduced pressure using rota flash evaporator until all the solvent had been removed. The yield of the extract was 12% w/w. when compared to the dried powdered material, which was then kept at -20°C until required.

**Animals**

C57 Black male mice, weighing 25-30 gm were used. Mice were obtained from the Animal house, K M C H College of Pharmacy, Coimbatore, Tamil Nadu. They were permitted food and water *ad libitum* up to the experimentation period. Prior to use, the mice were housed in polypropylene cages in group of six to eight animals under natural light-dark cycle. All the observations were made at room temperature in a noiseless diffusely illuminated room and were made between 9.00 to 17.00 h in the experimental room. All the experimental protocols were approved by Institutional Animals Ethics Committee (IAEC) as per provisions of Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) (KMCRT/PhD/ /2014-15), New Delhi, India.
Experimental Protocol

The following experimental method was followed to assess the locomotor behavioral effect of *B. sensitivum* (BS) on MPTP induced mice. 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP): 15 mg/kg of MPTP as a neurotoxin i.p. twice, 4 h apart in *vivo* and 20 µM in *vitro*. MPTP was dissolved in 0.9 % saline and administered i.p. Intraperitoneal injection of MPTP was given to Groups II, III, IV and V. Oral dosage of Carbipoda + Levadopa (Standard drug for Parkinson’s disease treatment) was administered to Groups III.

The animals were separated into six groups, each consisting of six mice.

1. Group I served as vehicle control (Distilled water)
2. Group II administered MPTP (20 mg/kg, i.p) (Sigma-Aldrich, Bangalore, India) four consecutive days,
3. Group III administered MPTP + carbidopa + levadopa (100 mg/kg, p.o)
4. Group IV administered MPTP + plant extract (250 mg/kg, p.o)
5. Group V administered MPTP + plant extract (500 mg/kg, p.o)
6. Group VI administered only plant extract (500 mg/kg, p.o)

Experimental Analysis

The actions of selected plant extract on natural locomotor activity were measured automatically using Actophotometer, (Medicraft photoactometer, model No: 600-40, S. No: PA-0149, India) four consecutive days,

Experimental Analysis

The actophotometer readings decreased 48.80 ±2.92 in the control animals (Group II) when compared to control group (Group I) (Table: 1).

Animals treated with standard drug showed a no significant difference in locomotor activity on the 0th day which then significantly increased in locomotor activity on the 7th day (61 ± 6.083), *(P < 0.001)*. Thus there was a significant increase *(P < 0.001)* in the locomotor activity of Group III when compared to Group I. Animals treated with the 1st dose (MPTP + *Biophytum* 250 mg/kg i.p.) showed a nonsignificant difference in locomotor activity on the 7th day (35.6 ±15.241, *(P < 0.05)*). Animals treated with *B. sensitivum* extract (500 mg/kg i.p.) also showed a highly significant activity on the 7th day (67.33 ±14.306, *(P < 0.001)*) when compared to negative control group (Group II) (Table 1). The present study showed that the *Biophytum* extract has considerable protection in MPTP induced hypolocomotion.

Elevated plus maze test

The entries of C57 black mice into dark are the normal character. The control group of mice shows more number of dark entries. The group treated with 500 mg of *B. sensitivum* extract shows significant increase in dark entries (157.83 ± 19.260), *(P<0.01)*. This proves that the motility of the animals is induced when the extract of *B. sensitivum* is administered (Table: 1). Administration of MPTP exhibits significant decrease (140.67 ±15.175) in dark entries as compared to Group I control. On the 7th day *Biophytum* extract along with MPTP administration mice showed less momentous difference *(P >0.001)*.

Hole Board

The normal, control group of animals show more number of dips into the hole board as an escaping mechanism. The group induced by MPTP shows less number of dips compared to control group (8.71 ±3.251). Table 2 shows that MPTP + 250 mg/Kg and MPTP + 500 mg/Kg dose of *B. sensitivum* extract showed propensity to increase the head dips, than the vehicle treated control group, although the result was not statistically significant. The 500 mg/Kg dose of *Biophytum* extract produced significant *(p<0.05)* increase in the head dips (13.50 ±4.416%), they show great enhancement and exhibit more number of dips more than that of MPTP and standard groups (12.00 ±13.675).

Rotarod Apparatus

Muscle rigidity of animals was evaluated by means of the rotarod apparatus. The mean fall-off time of vehicle treated control group (Group I) animals from the rotarod was 12.00 ±13.675. The mice treated with standard drug (10 mg/kg i.p.) also showed a significant increase *(P < 0.001)* on the 7th day (63.00 ±10.119), when compared to MPTP treated (Group II) animals on the similar day. Pretreatment with *B.
sensitivum along with MPTP (Group IV) at a dose of 250 mg/kg i.p. showed a rotarod reading 47.00 ±28.664 on the 7th day ($P < 0.05$). The dose of $B$. sensitivum extract (500 mg/kg i.p.) also showed a significant activity ($P < 0.01$) on the 7th day (104.5 ±4.150) as compared to Group II. A significant activity ($P < 0.001$) was observed on the 7th day (66.17 ±13.819) as compared to negative control group (Group II) (Table: 2).

**Step Down**
Effect of ethanol extract of $Biophytum$ on memory was observed using step down model in mice. Almost all the groups show similar results only. There is no much difference between the control group or induced group or treated group with $B$. sensitivum. The animals treated with levodopa and carbidopa (10 mg/kg i.p.) group significantly ($P < 0.001$) retained memory and increase in step down when compared to MPTP treated animals (Group II) on the 7th day (1.33 ±0.516). Administration of MPTP + $B$. sensitivum 500 mg/kg also exhibits similar results (Table: 2).

**Step Through**
Step through is a passive avoidance test. It is a simple and rapid test method for memory assessment (Das et al., 2000). On the 7th day MPTP administered mice showed significant variation and remarkable decrease in step through transfer latency (1.17 ±0.408), when compared to control (1.50 ±0.548) and $B$. sensitivum (500 mg/kg) ethanol extract treated mice (1.27 ±0.408) (Table: 2). Group V and Group VI animals were treated with alcoholic extracts of $B$. sensitivum extract shows restoration of locomotion compared to control group at the nonsignificant level.

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<th>Table 1: Effects of $Biophytum$ sensitivum ethanol extract in the Actophotometer test and Elevated plus maze test</th>
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Values are expressed as the mean ± S.D. Statistical significance ($p$) calculated by one way ANOVA followed by dunnett’s. ns- not significant $^*P<0.001$ $^**P<0.01$ $^***P<0.05$ calculated by comparing treated group with control group.

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<th>Table 2: Effects of $Biophytum$ sensitivum ethanol extract in the Hole Board test</th>
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**DISCUSSION**
Actophotometer is used for screening the locomotor and anti-anxiety activity in rodents, while the rotarod for muscle relaxant activity. Locomotor activity indicates attentiveness and the decline indicates sedative action. The GABA receptor compound is concerned in sedation, muscle relaxant and anxiety in CNS. Various neurological and mental disorders such as epilepsy, depression, Parkinson syndrome, Alzheimer’s disease are involved with this receptor. The increase in locomotor activity on alcoholic extract of $B$. sensitivum has shown stimulant effect in actophotometer. This provoked to evaluate it further, using paradigms of depression models. The EPM is commonly one of the most extensively used models of animal anxiety. An increase of the time and the fraction of the entrances into the open arms without altered locomotor activity are regarded as a dominant marker for an anxiolytic substance effect. Results showed that plant extract treated mice exhibited significant increase in the number of open arm entries. The number of arm entries, but
reduces in time spent in closed arm reflects plants anxiolytic property. Rodents exhibit anxiety and fear when to be found in a new environment and behavior could be determined through the inspection of rearing and grooming. With this hypothesis, present study showed ethanolic extract of \textit{B. sensitivum} at the doses of 500 mg produced significant anxiolytic action when compared to standard drug at a dose of 100 mg/kg. This may envisage that ethanolic extract of \textit{B. sensitivum} may interact with GABAergic transmission as diazepam and responsible for the anxiolytic action\cite{14}. The hole board test is helpful for modeling anxiety in animals, in this test an anxiolytic-like state may be reflected by an increase in head-dipping behaviors\cite{15, 16}. The mice when pretreated with \textit{B. sensitivum} (500 mg/kg, p.o.) for 7 days, significantly improved the number of dips and line crossings in hole board test, and this effect is comparable to that of levodopa group.

Rota rod test a standard animal model used to evaluate peripheral neuromuscular blockade and the motor coordination\cite{17}, a deficit in motor coordination would very likely affect performance in the behavioral tests. Rota rod test, the difference in the fall of time from the rotating rod between the vehicle and extract treated groups were taken as an index of muscle relaxation. Plant extract showed considerable decrease in the locomotory score and fall of time of the mice from the rotating rod. Passive-avoidance response (PAR) is widely used for the screening of drugs affecting learning and memory\cite{18}. The conventional and the most extensively employed parameters are step-down latency (SDL) and Step through test. The present study demonstrates that in a paradigm of short-term memory, \textit{B. sensitivum} extract produces development in passive avoidance acquisition and memory retrieval. The above results of behavioral tests are comparable with other preceding studies done on different parkinsonian animal models induced by MPTP.\cite{20, 21}. It has been hypothesized that antioxidants possibly will be neuroprotective in PD, by preventing neuronal death caused by intracellular free radicals\cite{22}. On treatment with alcoholic extract of \textit{B. sensitivum} reversed the behavior alterations induced by MPTP on 3rd day, but highest effect of extract was seen on 6th day of treatment. It might be due to occurrence of phytoconstituents like L-dopa, polyphenols and flavonoids\cite{23}.

**CONCLUSION**

The results of present experiments indicated that \textit{Biophytum sensitivum} extract attenuates MPTP-induced cognitive and behavioural impairments in mouse model of PD. Regulation of antioxidant defense mechanisms by OC may partially be responsible for its neuroprotective effect in MPTP-induced PD mice.

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