



MIMOSA PUDICA UP-REGULATES DAT AND TH PROTEINS EXPRESSION, IN TURN, LOCOMOTOR FUNCTIONS IN MOUSE MPTP-1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE MODEL OF PARKINSONISM

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ABSTRACT

Mimosa pudica is a traditional Siddha medicinal plant useful in the treatment of various neurological (vaatham) disorders. The main objective of the study is to demonstrate the neuroprotective effects of *Mimosa pudica* on against MPTP induced Parkinsonism in mice model. Male C57BL/6J mice (20-25 g bwt) were used for the study. Following acclimatisation the animals were divided into five groups with 6 in each. Group served as I negative control, Group II served as MPTP group, Group III as carbidopa 250 mg/kg IV & V as *Mimosa pudica* at 100 and 300 mg/kg, respectively. Animals were pretreated with vehicle or drugs (once a day) for five consecutive days. On day 5, one hour after vehicle or drug administration, MPTP was injected intraperitoneally at 80 mg/kg b.wt in two divided doses (2 X 40 mg/kg bwt. at 16 h interval). Forty eight hour after MPTP, animals were assessed for motor functions using horizontal grid test and vertical grid test. Then the animals were euthanised and brains were collected and processed for immunohistochemical analysis of dopamine transporter (DAT), tyrosine hydroxylase (TH) and α -synuclein (α -syn) expressions. *Mimosa pudica* improved DAT and TH expressions. α -syn expression was significantly decreased in *mimosa* treated animals when compared to vehicle treated animals. The results indicate *mimosa pudica* is a potent neuro-protective agent against MPTP induced Parkinsonism.

KEYWORDS: *Mimosa pudica*, Siddha medicine, TH-Tyrosine hydroxylase, DAT-Dopamine transporter, MPTP-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, α -synuclein



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INTRODUCTION

Siddha system of medicine is one of the oldest traditional systems of medicines in the world and it was developed by a lineage of 18 Siddhars and their successors for treating various diseases of mankind¹. Its holistic approach focuses on the balanced importance between physical and mental wellbeing and appropriate medicines with life style modifications. According to Siddha system of medicine, Parkinson's disease can be correlated to "Sira-kamba Vatham" or "Naddukku Vatham" or "Paanikamba vatham", due to the disorder of vatham humour. Vitiating of vatham humour leads to Parkinson's clinical symptoms like muscular rigidity and postural instability, resting tremor, bradykinesia, etc.,². Various factors like environmental, chemical and drugs, head trauma, genetic factors are shown to precipitate Parkinson's disease. Pathophysiological reports mark degeneration of dopaminergic neurons which are densely populated in substantia nigra and corpus striatum and are linked with motor dysfunctions and cognitive impairment as well³. However, the loss of non-dopaminergic and associated non-motor functions are results of alterations in the multiple neurochemical mechanisms⁴. The non-motor PD symptoms include depression, anxiety, and cognitive decline⁵. The link between the dopamine-dependent oxidative stress and microglial activation, leading to chronic inflammatory state in PD is well established. Treatment of PD with conventional drugs produces only a symptomatic relief. However due to the associated oxidative stress and inflammation, these treatments may not be complete. Many herbal-based drugs were shown to possess significant neuro-protective effects in PD.⁶ *Mimosa pudica* (Family: Mimosoideae) a creeping annual or perennial herb grows abundantly in India. In Siddha system it is called as "*Thottar Chinungi*" mainly used to treat hyperglycaemia, chronic ulceration and loss of libido. It is indicated to neutralize vitiating vaatham and used in the treatment of "Odu Vaatham" in Siddha⁷. The anti-vaatham property of *mimosa pudica* may be the factor for considering its use to treat PD. Our earlier in vitro study showed neuro-protective action of MP against MPP+ induced neurotoxicity in SHSY5Y cell lines. The present work is undertaken to demonstrate the neuro-protective effects of *mimosa pudica* against MPTP induced toxicity in mice model.

MATERIALS AND METHODS

Chemicals and Reagents

Whole plant raw powder of *mimosa pudica* was procured from M/s. Arogya Health Care Pvt. Ltd Chennai (MUG/2725/16-17). MPTP, mouse anti-TH, mouse anti- α synuclein, rat anti-DAT, and anti-mouse IgG were purchased from Sigma Aldrich, USA. Immuno-Cruz mouse ABC Staining kit was procured from Santa Cruz, USA. All the other chemicals and reagents used were of analytical grade.

Animal husbandry

Male C57BL/6J mice (20-25 g b wt) were obtained from Biogene, Bengaluru (Breeder Reg. No. 971/bc/06/CPCSEA) and were acclimatized to laboratory conditions for seven days prior to experiment.

Six animals per cage were housed in a polypropylene colony cages in a good-ventilated room at a temperature $20 \pm 5^\circ\text{C}$ and 30-70% relative humidity, with a 12-h light/dark photoperiod. Animals were fed with standard rodent maintenance diet (M/s. Kamadhenu. Ltd, Bengaluru) and purified water *ad libitum*. Institutional Animal Ethics Committee (IAEC), JSS College of Pharmacy, Mysuru, approved the study.

Experiment Design

Following acclimatization, mice were pre-trained for vertical grid test until ceiling performance was reached. Animals were divided into five groups with 6 in each. Group I received 0.5% CMC + saline (Negative control), Group II received 0.5% CMC + MPTP (MPTP group), Group III received carbidopa (250 mg/kg, p.o) + MPTP, IV & V received *mimosa pudica* at 100 and 300 mg/kg, p.o, respectively + MPTP. Animals were pretreated with vehicle or *mimosa pudica* or carbidopa (once a day) for five consecutive days. On day 5, one hour after vehicle or test drug administration, MPTP was administered intraperitoneally at 80 mg/kg b.wt in two divided doses (2 X 40 mg/kg b.wt. at 16 h interval). Forty eight hour after MPTP administration, animals were assessed for motor functions using horizontal grid and vertical grid tests. Following the motor function tests, animals were perfused using 4% paraformaldehyde, brains were excised out substantia nigra pars compacta (SNpc; ~ Bregma - 3.16 mm, interaural 0.64 mm) region was identified using Paxinos and Franklin mouse brain atlas (Paxinos and Franklin, 2001) and isolated. For Immunohistochemistry, whole brain was fixed in 10% neutral buffered formalin for 48 h; sectioned and stained for protein expressions. Dose of *mimosa pudica* was fixed based on the acute toxicity⁸.

Horizontal Grid Test⁹

Horizontal grid test was performed according to Kim et al., (2010) method with slight modifications. Apparatus consisted of horizontal grid mesh (total size 12 cm²: openings 0.5 cm²) mounted 20 cm above a hard surface, thus discouraging falling, but not leading to injury in the case of falling. Mice were placed individually in the centre of the horizontal grid and supported until it grabs the grid with both fore and hind paws. Grid was then inverted so that the mouse hangs upside down and they were monitored for hanging time.

Vertical Grid Test⁹

Vertical grid test was performed according to the method of Kim et al. (2010) with slight modifications. Vertical grid apparatus consisted of an open box of 8 X 55 X 5 cm set. The backside of the vertically standing box was made of a wire mesh, the front side was open and the other four sides were made of black plexiglass. Mice were placed individually inside the apparatus at 3 cm from the bottom, facing upwards and allowed to climb up the grid. Prior to MPTP administration, the mice were acclimatized to the vertical grid thrice a day for 2 days. Trial was repeated whenever the mice failed to climb up within 60 s. The same trial was made 48 h after first MPTP injection and parameters such as the time to climb up the grid and immobility period were recorded.

Immunohistochemistry

Serially sectioned five micrometer thickness of SNpc region was fixed in 3-aminopropyltriethoxilane coated slides. The slides were deparaffinized in xylene and rehydrated in a graded series of ethanol and distilled water. Then the slides were boiled in citrate buffer (10mM, pH 6.0) for 20 min at 90°C for antigen retrieval. To remove the endogenous peroxidase activity, they were incubated with 3% hydrogen peroxide (H₂O₂) for 15 min at room temperature. Then the slides were placed in blocking buffer containing 1% bovine serum albumin (BSA) with 0.1% Tween-20 in 1X PBS (pH 7.4) for 30 min at 37°C. During each and every step, the slides were washed at least three times with 1X PBS. Then the sections were incubated for 24 h with primary antibody: rabbit polyclonal TH (1:100), mouse monoclonal SYN (1:100), rat monoclonal DAT (1:50) in 1% BSA, 0.1% Tween-20 and 0.02% sodium azide in TBS. After washing with BSA in TBS, the sections were incubated in anti-rabbit IgG (1:300), anti-mouse IgG (1:100), anti-rat IgG (1:100) secondary antibody in BSA for one hour. Sections were washed with PBS and exposed to ABC. Then slides were stained using AEC

staining for 10 minutes. All slides were counterstained with Mayer's hematoxylin and visualized in light microscope. The numbers of TH, SYN and DAT immune reactive cells were counted. Cell counts were determined every sixth section (total 8–10 sections) through SNpc corresponding to ~ - 3.16 mm bregma, ~ 0.64 mm interaural co-ordinates from each of the animals, and three animals/group were used for cell counts. Percentage immune positive cells were calculated as: (Number of immune positive cells/Total number of cells) X 100. The cell counts were then averaged for each animal and these averages were used to calculate a mean ± SEM for each treatment group.

Data Analysis

Data were expressed in mean ± standard error of mean (SEM). Mean difference between the groups were analysed using one way ANOVA followed by Tukey's multiple comparison test as post hoc in GraphPad Prism 5.0. p value less than 0.05 was considered to be statistically significant.

RESULTS

Vertical grid test

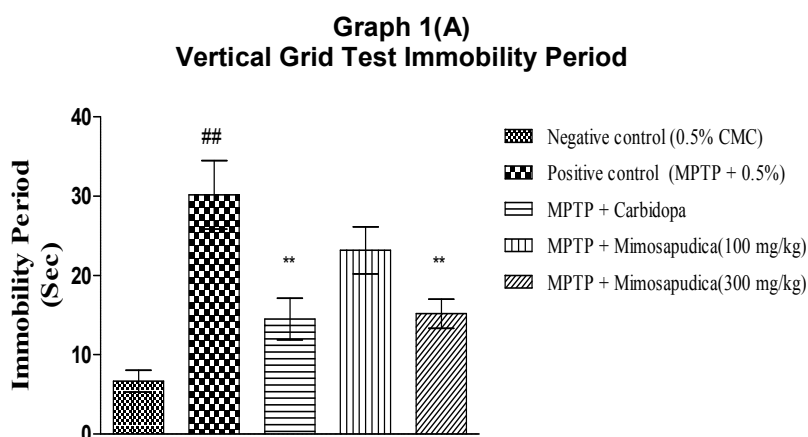


Figure 1

MPTP mice took longer time to climb the grid [$F(4,25) = 6.121, p < 0.01$] with increased immobility period [$F(4,25) = 10.16, p < 0.01$] when compared to control mice [Graph1(A)]. Mimosapudica at 300 mg/kg, p.o significantly decreased the time taken ($p < 0.01$) and immobility period ($p < 0.01$) when compared to MPTP mice and the values were found to be comparable with that of Carbidopa [Graph1].

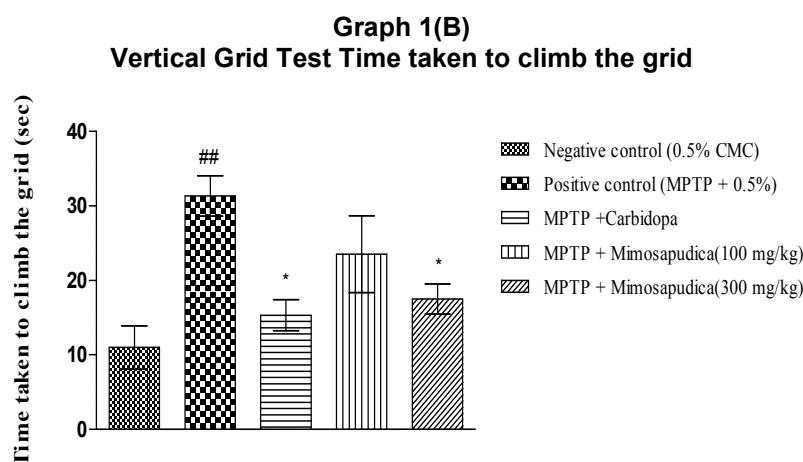


Figure 2

Effect of Mimosapudica on vertical grid test in MPTP induced mice. (A) Immobility period (sec) and (B) Time taken to climb the grid (sec), Values were expressed in Mean ± SEM, n = 6 animals/group, Statistical analysis was performed using one way ANOVA followed by Tukey's multiple comparison test, ## indicates p value < 0.01 Vs group I, *,** indicates p value < 0.05 and 0.01 respectively Vs group II.

Horizontal grid test

MPTP mice showed a significant increase in hang time [F (4,25) = 10.66, p<0.01] when compared to control mice in horizontal grid test. Oral administration of *Mimosa pudica* produced a dose dependent 300 mg/kg, p.o (p<0.01) decrease in the hang time in comparison to MPTP mice [Graph 2].

Graph 2
Horizontal Grid Test Hanging Time

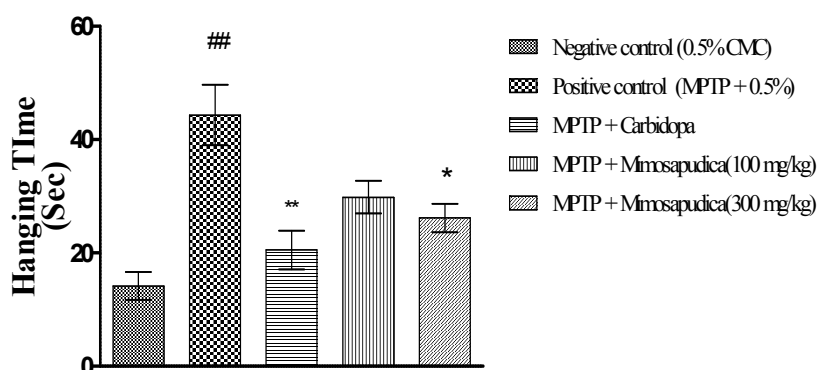


Figure 3

Effect of *Mimosa pudica* on horizontal grid test in MPTP induced mice. Represents hang time (sec), Values were expressed in Mean ± SEM, n = 6 animals/group, Statistical analysis was performed using one way ANOVA followed by Tukey's multiple comparison test, ## indicates p value < 0.01 Vs group I, *,** indicates p value < 0.05 and 0.01 respectively Vs group II.

Immunohistochemistry

MPTP treated mice showed significant increase in SYN positive cells [F(4,45) =9.446, p<0.01] and significant decrease in DAT and TH positive cells [F(4,45) = 10.23, p<0.01 and F(4,45) = 6.811, p<0.01, respectively], in SNpc region when compared to control mice. Animals treated with *Mimosa pudica* 300 mg/kg) showed decreased SYN and increased DAT and TH positive cells (p<0.01) when compared to MPTP mice [Graph 3(A),3(B),3(C)].

Graph 3 (A)
No of DAT positive cells (%)

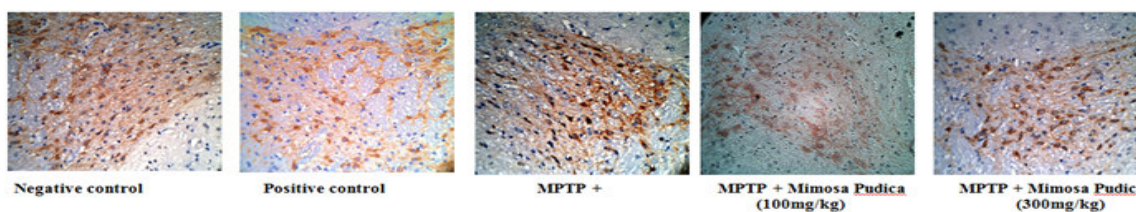
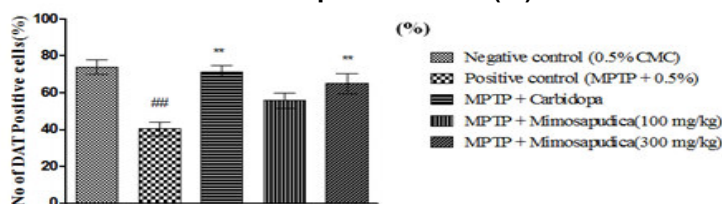


Figure 4

Graph 3 (B)
No of TH positive cells (%)

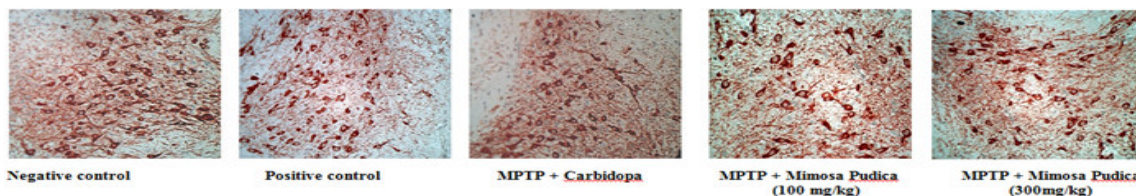
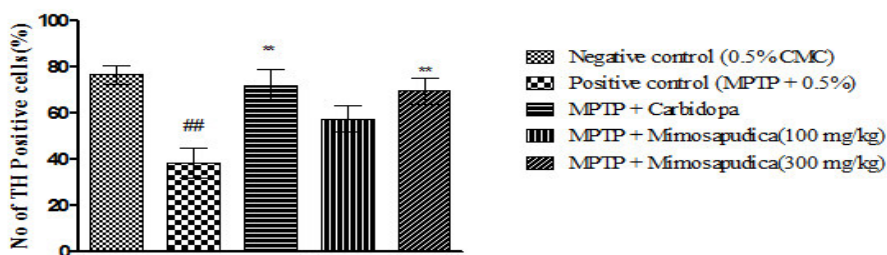


Figure 5

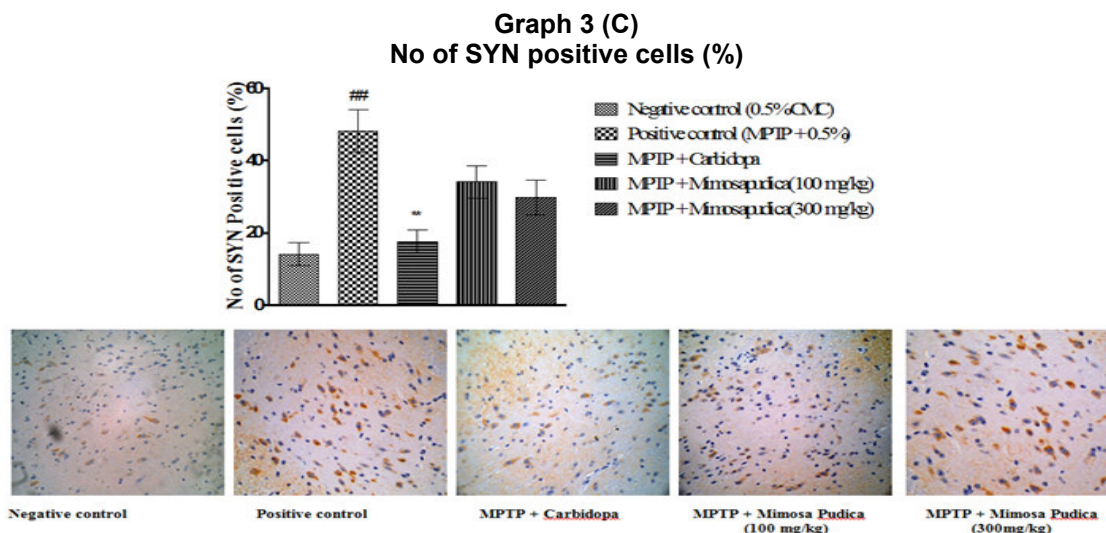


Figure (4),(5),(6) Effect of *Mimosa pudica* on DAT,TH,SYN protein expression by Immunohistochemistry. Values were expressed in mean \pm SEM. Statistical analysis was performed using one way ANOVA followed by Tukey's multiple comparison test, ## indicates p value <0.01 Vs group I, **indicates p value <0.01 Vs group II

DISCUSSION

The present study demonstrates the neuro-protective effects of *mimosa pudica* in mice MPTP model of Parkinsonism. Toxicants provoked Parkinson's disease in animal models mimic clinical pathogenesis wherein the changes at misfolding and/ aggregation of proteins those participate in dopaminergic neuronal death and secondly the disease aggravating factors like oxidative stress, neuronal inflammation and necrotic injuries¹⁰. 1-methyl- 4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induced Parkinson's diseases is one of the mostly used mouse models in PD research. MPTP, a highly lipophilic agent, on systemic administration penetrates blood brain barrier easily and it is metabolised by MAO-B enzyme (present in glial and serotonergic cells) to MPP⁺, which is a highly hydrophilic compound¹¹. MPP⁺ is actively taken up by dopamine transporter (DAT) into dopaminergic neurons wherein the toxicant damages the mitochondrial structures and depletes energy synthesis and also triggers oxidative stress¹². The energy crisis leads to decreased dopamine production which in turn leads to motor dysfunction in MPTP intoxicated animals¹³. On the other hand, MPTP is shown to accumulate and nitration of α -synuclein in SNpc dopaminergic neuronal cells¹⁴. Because of these properties, MPTP is considered as a gold standard model for preclinical screening in new drug discoveries which are aimed at alleviating PD symptomatically. In the present study, administration of MPTP, up-regulated the protein expression of α -synuclein and down-regulated DAT and TH expressions in vehicle treated mice group. These animals displayed severe motor deficits in vertical and horizontal grid tests. These animals also showed decreased feed intake and water intake (data not shown). Pre-treatment with *mimosa pudica* improves DAT and TH expressions. α -synuclein expression was significantly decreased in mimosa treated animals when compared to the vehicle treated animals. Our recent in vitro study showed the neuro-protective potential of mimosa against MPP⁺ induced damages in SHSY5Y cell lines¹⁵. Mimosa is also shown to possess neuro-protective effects against chemicals induced epilepsy, adaptogenic and nootropic activities¹⁶.

^{17, 18, 19}. These reports reveal the CNS selective actions of mimosa and the ability of the chemical principles present in mimosa to penetrate the blood brain barrier and exert neuro-protective actions. However, till date there is no data available on the pharmacokinetic and bioavailability either in preclinical or clinical set up. The major chemical principles in mimosa include the presence of alkaloids, non-protein amino acid (mimosine), flavonoids C-glycosides, sterols, terpenoids, tannins, and fatty acids²⁰. However, at this stage it is unclear to find out the corresponding active principle(s) responsible for the observed neuro-protective effects against MPTP induced damage. Mimosa is used in Siddha system of medicine to encounter vaatham and vaatham associated ailments. In accordance with Siddha system of medicine, derangement of vatham humour produces tremor, rigidity, difficulty in walking and swallowing food, cognitive decline, sleep disorders etc. These clinical signs can be correlated to features of PD. The pathophysiology and modern clinical description of PD were established in late 1850's, however, in spite of lack of modern scientific tools, Siddha system clearly described PD as a disorder of central nervous system many thousand years back and interestingly, the therapeutic regimen and life style conditions were also established in Siddha for PD management. Apart from internal medicines, the external therapies are also important in Siddha practice in the management of PD with the aim to improve the muscular functions.

CONCLUSION

Mimosa pudica is a potent neuro-protective agent against MPTP induced neuronal degeneration and a potential candidate for further investigation on the molecular mechanism and to be lead molecular in PD drug discovery.

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ABBREVIATIONS

PD – Parkinson's disease, ST – Striatum, SNpc – Substantia nigra pars compacta, SYN - α -synuclein,

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TH - Tyrosine hydroxylase, DAT – Dopamine transporter, MPP⁺ - 1-methyl-4-phenylpyridinium, MPTP- 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, BSA- Bovine serum albumin, MAO-Mono amine oxidase, SEM – Standard error of the mean.

CONFLICT OF INTEREST

Conflict of interest declared none.

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