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Original Article

MIMOSA PUDICA EXERTS NEUROPROTECTION AGAINST MPP+INDUCED NEUROTOXICITY IN SHSY5Y CELL LINES-AN *IN VITRO* MODEL OF ANTI-PARKINSONISM

MAHADEVAN M. V.[1], RAMASWAMY R. S.[2*], BANUMATHI V.[3]

[1]Sirappu Maruthuvam Department, National Institute of Siddha, Chennai 600047, India, [2]Director General, Central Council for Research in Siddha, Ministry of AYUSH, Govt of India, Govt Anna Hospital campus, Arumbakkam, Chennai 600106, India, [3]Director, National Institute of Siddha, Chennai 600047, India
Email: dr.rsramaswamy@gmail.com

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ABSTRACT

Objective: Parkinson's disease (PD) is one of the of a neurodegenerative disorder, It's decreased the dopaminergic neurones, tyrosine hydroxylase (TH) and increased the α-synuclein protein level. This study was conducted to investigate the neuroprotective effect of *Mimosa pudica* have the abilities to improve TH and DAT proteins expression against MPP^+ induced neurotoxicity, in *in vitro* model of Parkinson's disease using SH-SY5Y human neuroblastoma cell lines.

Methods: *Mimosa pudica* were pre-treated with various concentration for cell viability assay. Vehicle alone or *Mimosa pudica* (300µg) for 24 h, and then were co-treated with 1000µM MPP^+ for 15 min in the continued presence of vehicle or *Mimosa pudica*. After treatment, cells were collected for protein expression.

Results: Cell viability assay confers the inhibitory concentration cell death of *Mimosa pudica*. MPP^+ significantly down-regulated the protein expression of TH ($p<0.01$) and DAT ($p<0.05$). *Mimosa pudica* decreased the expression of α synuclein ($p<0.01$) in MPP^+ intoxicated cell lines.

Conclusion: The present study showed that *Mimosa pudica* exerts neuroprotection by suppressing α synuclein and the dopaminergic neurdegeneration. *Mimosa pudica* may be due to quecertin which might be acted via the anti-oxidant mechanism. The above finding suggests that *Mimosa pudica* may act as a potential target in the management of PD.

Keywords: Parkinson's disease, *Mimosa pudica,* Siddha medicine, SH-SY5Y, MPP^+, α synuclein, Tyrosine hydroxylase, Dopamine Transporter

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INTRODUCTION

Siddha system of medicine, which is practised prevalently in the southern part of India, especially in Tamil Nadu, is familiar among Tamil-speaking people and outside of the landscape too. The name Siddha medicine owes its origin to medicinal ideas and practices rendered by sages called Siddhar's/"Holy immortals". Siddha system of medicine is established mainly with 18 Siddhas and the most renowned are Agathiyar, Thiru moolar and Bhogar [1].

Parkinson's disease (PD) is one of the of neurodegenerative disorders characterized by paucity and slowness of the movement (bradykinesia), tremor at rest, rigidity, shuffling gait and flexed posture. Decreased levels of dopaminergic neuronal density in the substantia nigra (SNpc) and striatum (ST) and more importantly tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine synthesis, are major biochemical indications in PD pathology. Remarkable impeachment of α-synuclein (SYN) provokes Lewy body (LB) pathologies that involve the deposition of LBs in cell bodies. Up-regulation of SYN was shown to trigger the generation of TNF-α and IL–1β in cultured Neuronal cell lines [2, 3]

Commonly vaatha diseases mentioned in Siddha are correlated to neurological disorders in modern medicine parallel with Siddha system. In Siddha, the vaatha diseases (vitiated vaatha humour) like Paanikamba vatham, Sirathamba vatham and Nadukku Vaatham wherein the patients clinically express difficulty in walking, resting tremor and loss of sensation (chronic status) in hands and feet, rigidity, and sleeplessness reflects the features of Parkinson's disease [4]. These notions ascribe to the existence of medical knowledge and diagnostic procedures of PD were in Siddha even before the scientific demonstration of PD. The clinical correlation in both Siddha and modern medicine demonstrates the motor and cognitive dysfunctions in PD. In Siddha, treatment of PD is basically aimed at restoring vitiated vaatham by external and internal therapies. Major herbs and herbo-mineral preparations used includes *Mucuna pruriens*, *Ulunthu thylum*, (5) and *Kalamega Narayana chendooram,* [17] etc., which are shown to restore vitiated vaatham and thereby motor functions in PD [5]. In Siddha medicine, *Mimosa pudica* (Fam: Fabaceae) is indicated to treat diabetes mellitus, chronic wounds and impotency. *Mimosa pudica* possesses hypnotic action which shows its ability to penetrate the blood-brain barrier. *Mimosa pudica* relives "Odu vaatham" a kind of vaatha disease [6]. Based on the traditional clinical indication, the present study was performed to understand the neuroprotective activity of *Mimosa pudica* in *in vitro* model of PD using SHSY5Y human neuroblastoma cell lines. The study reveals that *Mimosa pudica* have the abilities to improve TH and DAT proteins expression against MPP^+ induced neurotoxicity, an *in vitro* model of PD.

MATERIALS AND METHODS

Chemicals and reagents

Entire plant raw powder of *Mimosa pudica* was procured from M/s. Arogya Health Care Pvt. Ltd Chennai (MUG/2725/16-17). SHSY5Y human neuroblastoma was procured from NCCS, pune, india. MPP^+iodide, 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 and were obtained from SISCO Research Laboratories Pvt Ltd. Mumbai, India.

Standardisation of *Mimosa pudica* by HPTLC

Mimosa pudica was subjected to basic phytochemical analysis and major secondary metabolites such as tannins, flavonoids, and total phenols content were estimated following standard protocols. *Mimosa pudica* was standardised for quercetin content in HPTLC using silica gel GF254 as the stationary phase and chloroform: ethyl acetate: formic Acid: MeOH (3: 3: 0.4:0.1) as mobile phase [18]. Spots were developed in ascending mode and scanned at 412 nm.

Mimosa pudica (10 mg/ml) and quercetin (100 µg/ml) were prepared in methanol.

Cell culture maintenance and treatment

Human neuroblastoma SH-SY5Y cells (NCCS, Pune), possess morphological, biochemical, and electrophysiological characteristics of dopaminergic neurones and have been widely used in the study of cell model for PD [7]. Cells were cultured in DMEM+F12 supplemented with 10% (v/v) heat-inactivated foetal calf serum and 100 units/ml penicillin/streptomycin. Cells were kept at 37 °C in humidified 5% CO_2 and 95% air. All experiments were carried out 24–48 h after cells were seeded. The cells were pre-treated with vehicle alone or *Mimosa pudica* (300µg) for 24 h, and then were co-treated with 1000µM MPP^+ for 15 min in the continued presence of vehicle or *Mimosa pudica.* A pilot experiment was carried out with various concentrations of *Mimosa pudica* using cell viability as the end point and 300µg *Mimosa pudica* provided the maximum reduction in cell death (data not shown) hence further studies were carried out using 100 and 300µg of *Mimosa pudica.*

Cell viability or MTT assay

SHSY5Y cells were seeded in 96-well plates at a density of 8,000 cells/200µl/well for 24 h. Cells were treated with *Mimosa pudica* (1-1000µg/ml), and incubated at 37 °C for next 24 h. At 20 h following mimosa treatment, cells were incubated with 5 mg/ml MTT for 4 h. At the end of the experiment, the medium was removed, the insoluble formazan product was dissolved in DMSO (100 µl) and kept in the dark for 15 min. The intensity of purple colour developed was measured at 570 and 630 nm. Inhibitory concentration 50 (IC50) of *Mimosa pudica* was calculated using the formula:

% Growth inhibitory rate = ([Control OD–Test OD]/Control OD)*100

Western blot analysis

SHSY5Y were seeded in 6 well poly-D-lysine precoated plates (25 µg/ml) at a density of $1X10^6$ cells/well and allowed to grow for a period of 48 h. Sterile filtered *Mimosa pudica* or vehicle will be added to the pre-fixed wells and incubated for 24 h. 1000 µM/ml of MPP+ was added to respective wells and incubated for 15 min to induce neurotoxicity. Following incubation, all the wells will be refreshed with media and left overnight. Cells were lysed with 0.1 ml lysis buffer (1% NP40; 50 mmol Tris-HCl, pH 7.6; 5 mmol EDTA), followed by 30 min incubation on ice. The lysate was centrifuged at 15,000 g for 10 min at 4 °C. The supernatant portion (total lysate) was collected, and protein levels were determined. Samples containing 40 µg protein were used to separate SDS-PAGE (100V) and transferred to PVDF membrane (230mA for 90 min). Membranes were blocked with 5% milk for 1 h and washed three times with tris-buffered saline for 5 min each. Primary antibody (TH, DAT and α-synuclein) diluted in 2% BSA was added to the membrane and incubated overnight at 4 °C. The membrane was washed thrice with TBST for 5 min each. The secondary antibody diluted in 2% milk was then added to the membrane and incubated for a period of 1 h and washed with TBST. The bands were visualised with ECL solution [19].

Data analysis

Data were expressed as mean±SEM. The mean difference between the treatments was analysed by one-way ANOVA followed by Tukey's multiple comparisons as a posthoc test. $p<0.05$ is considered as significance criterion. Statistical analysis was performed using GraphPad Prism 5.0 version, USA.

RESULTS

The present study demonstrated the neuroprotective effect of *Mimosa pudica* against MPP^+ induced neurotoxicity in SHSY5Y cell lines.

Standardisation of *Mimosa pudica*

Basic phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins and total phenols in the aqueous extract of *Mimosa pudica*. Flavonoids, tannins and total phenolic contents of *Mimosa pudica* were found to be 19.70±1.92, 25.63±0.49 and 93.32±5.73, respectively. Quercetin content in methanolic extract of *Mimosa pudica* was found to be 0.20±0.03% w/w. Chromatogram of standard quercetin and *Mimosa pudica* were shown in fig. 2.

Cell viability assay

IC_{50} value of *Mimosa pudica* (concentration of extract required to cause 50% cytotoxicity or cell death) was calculated from regression equation prepared from concentrations versus cytotoxicity. IC_{50} value of *Mimosa pudica* was 211.05±3.65 in the tested conditions (fig. 1A). The higher IC_{50} value indicates the non-toxic nature of *Mimosa pudica* to SHSY5Y cell lines.

In vitro* neuroprotective effects of *Mimosa pudica

In vehicle-treated cells, MPP^+produced significant morphology changes like cell shrinkage, loss in membrane structure and loss in cell number (fig. 1B). Treatment with *Mimosa pudica* restored the cell structure and increased the cell viability by alleviating MPP^+ induced neurotoxicity in SHSY5Y cell lines.

Treatment with MPP^+ significantly down-regulated the protein expression of TH ($F(3,8)=26.48$, $p<0.01$) and DAT ($F(3,8)=13.35$, $p<0.01$) when compared to vehicle treated cell lines. MPP^+ significantly up-regulated α-syn ($F(3,8)=10.80$, $p<0.01$) expression when compared to vehicle treated cell lines. Treatment with *Mimosa pudica* significantly up regulated TH ($p<0.01$) and DAT ($p<0.05$) and down-regulated α-synuclein ($p<0.01$) expression in MPP^+ intoxicated cell lines **(**fig. 3**)**. These data reveal the neuro-protective potential of *Mimosa pudica* against MPP^+ induced toxicity in SHSY5Y cell lines.

DISCUSSION

Parkinson's disease is a debilitating and progressive neuro-degenerative condition, wherein till date the treatment strategies focus only on the symptomatic relief. Various classes of drugs such as dopamine agonist, dopamine replenishment therapy and monoamine oxidase inhibitors produce severe side effects and the sensitivity for the therapy goes low on long-term exposure. Yet, there is continuous efforts in the development of new drug therapies for the management of PD. Herbal based drugs offer substantial protective effects in the long-term management of various diseases including neurological disorders. *Mimosa pudica* was shown to have neuroprotective potential using various animal models of neurological disorders.

The mechanism of MPP^+ induced neurotoxicity is largely mediated via mitochondrial dysfunction. MPP^+ enters dopaminergic cells through dopamine transporter (DAT), and inhibits complex I in mitochondrial electron transport chain [8]. This decreases ATP production and triggers the generation of oxygen species (ROS) and apoptosis leading to neuronal death [9]. These data are consistent with the present study observation, wherein MPP^+ decreased DAT and TH expression, indicating dopaminergic neuronal death, which may be possible due to the accumulation of cytokines and oxidative stress. *Mimosa pudica* possesses wider pharmacological activities [10] and in particular, it is shown to exert neuroprotective activity such as anticonvulsant [11], anti-anxiety, anti-depression, adaptogenic and nootropic activities [12, 13]. These data demonstrated the neuronal reach of the active principles present in *Mimosa pudica*, in particular, tannins, flavonoids and total phenols. In the present study, *Mimosa pudica* was standardised for quercetin content which was found to be 0.20±0.03% w/w of aqueous extract. This is performed to ensure minimally or no batch to batch variation in the active principles present in *Mimosa pudica* keeping quercetin as a chemical marker. Quercetin was also shown to have anti-Parkinson's [14], anti-Alzheimer's [15], neuroprotective activity in cerebral ischemia and anti-neuro-inflammatory activity [15, 16].

Exposure to *Mimosa pudica* improved the TH and DAT expression and decreased α-syn in the MPP+ intoxicated cell lines. This may be corroborated to protective effects against MPP^+ triggered free radical generation. Further, quercetin is also shown to possess substantial antioxidant activity [12]. *Although at this stage, the mechanism of action of Mimosa pudica and active principles involved in the neuroprotective activity are not clear, in the present study, it*

(the anti-Parkinson's activity) may be due to quercetin which might act via the antioxidant mechanism. Our lab is involved in further studies to identify the active principles and to understand the mechanism of action of *Mimosa pudica.*

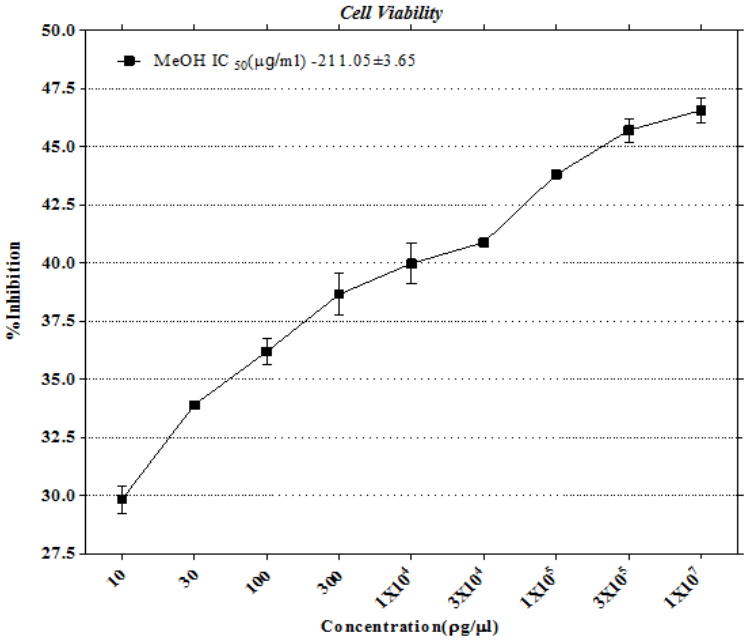

Fig. 1(A): % inhibition of cell viability *Mimosa pudica* the values are expressed in pictogram

Drug treatment

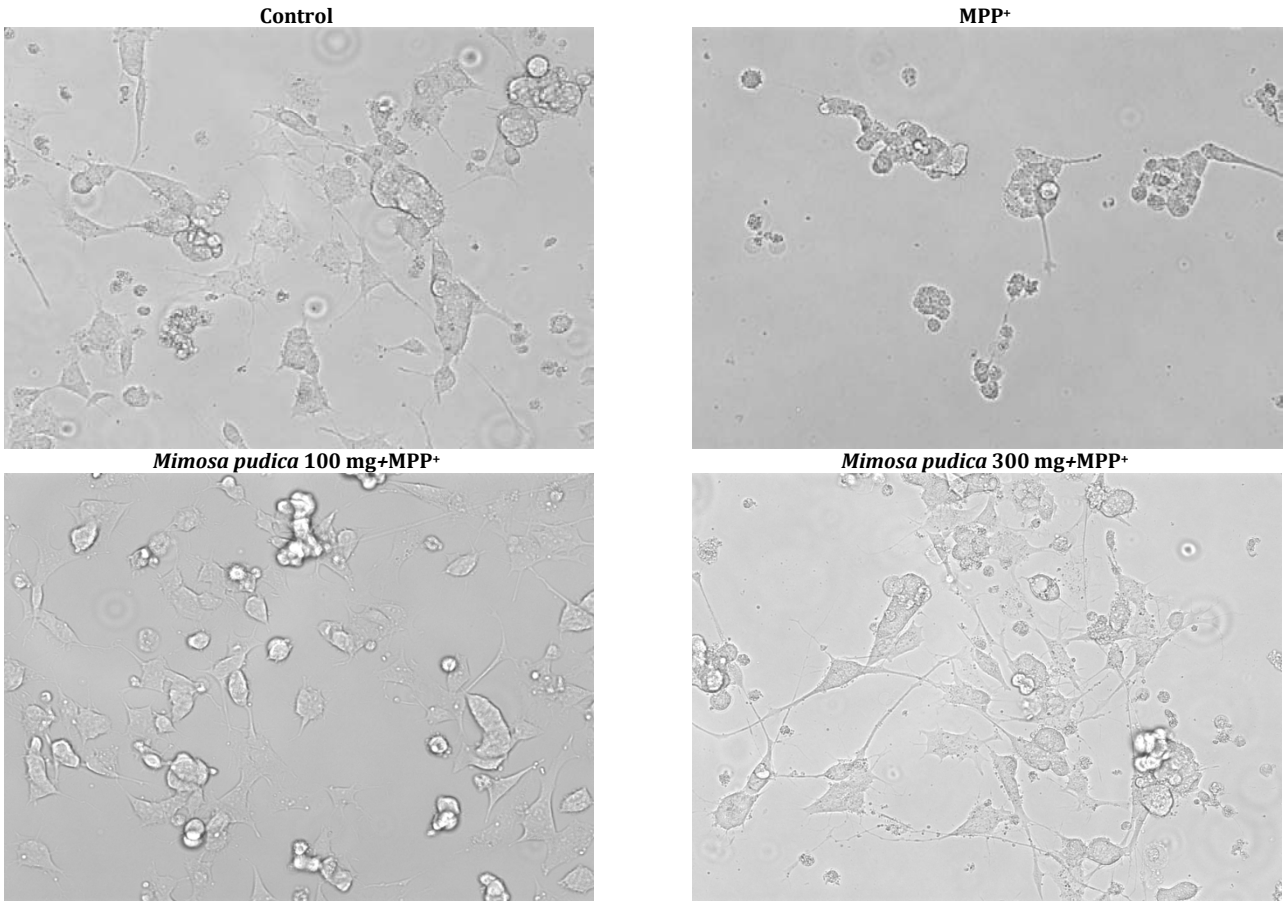

Fig. 1(B): Various Treatment of *Mimosa pudica* and MPP+. MPP+ treated cells shows cell shrinkage and Mimosa treated cells shows protection on neurons

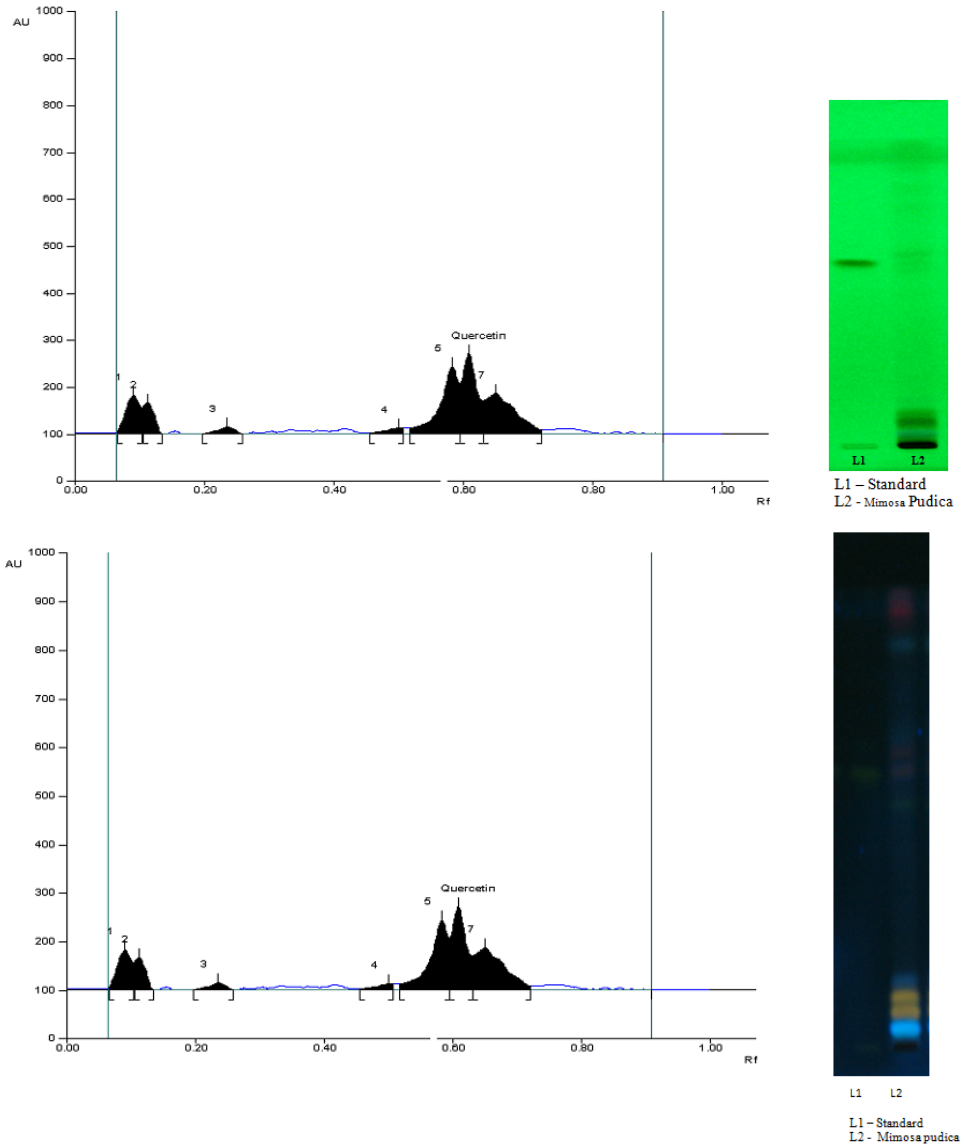

Fig. 2: Chromatogram shows Quercetin content of *Mimosa pudica* extract and standard quercetin

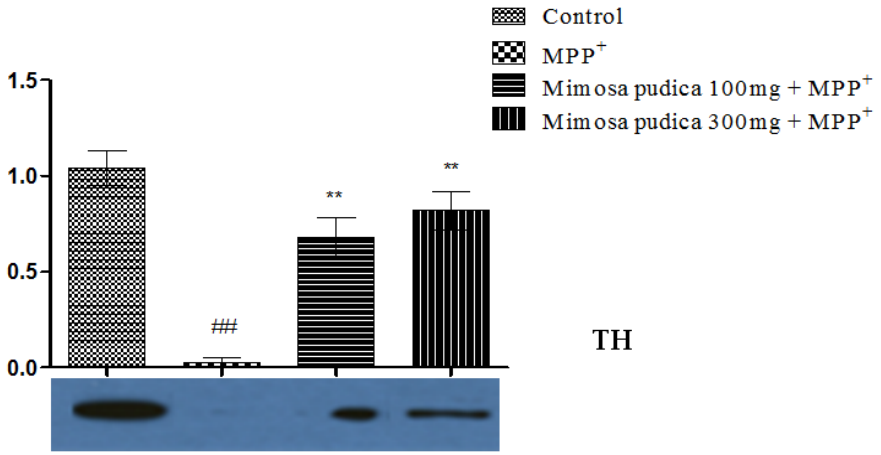

Fig. 3(A): Effect of *Mimosa pudica* on TH protein expression in MPP+ Treated cells. Values were expressed in mean±SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison tests; ## indicates p value<0.01 Vs group I, **indicates p value<0.01 Vs group II

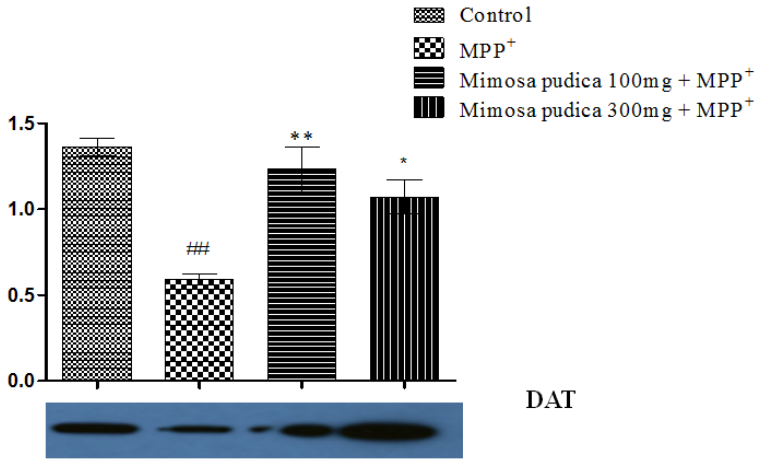

Fig. 3(B): Effect of *Mimosa pudica* on DAT protein expression in MPP⁺Treated cells. Values were expressed in mean±SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison tests, ## indicates p value<0.01 Vs group I, *,indicates p value<0.05 and 0.01 Vs group II**

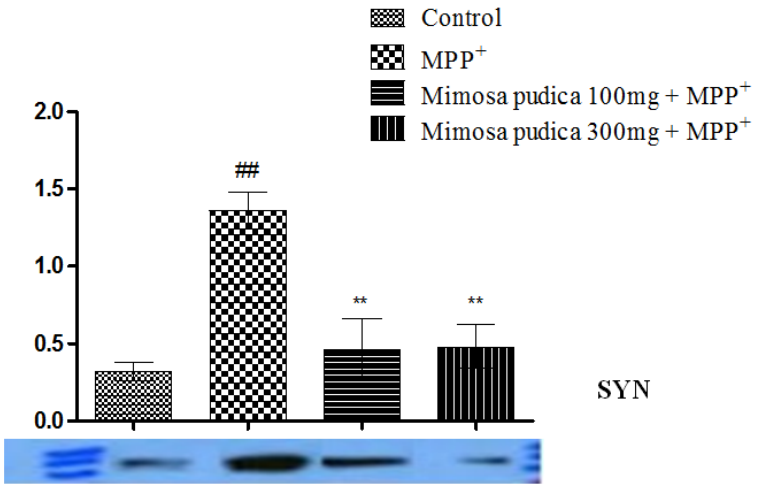

Fig. 3(C): Effect of *Mimosa pudica* on SYN protein expression in MPP⁺Treated cells. Values were expressed in mean±SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison tests; ## indicates p value<0.01 Vs group I, **indicates p value<0.01 Vs group II

CONCLUSION

Mimosa pudica possesses anti-Parkinson's activity, which may be corroborated by its antioxidant principles, at least partly due to quercetin.

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ABBREVIATION

PD–Parkinson's disease, ST–Striatum, SNpc–Substantia niagra pars compacta, SYN-α-synuclein, TH-Tyrosine hydroxylase, DAT–Dopamine transporter, TNF–α–Tumor necrosis factor, IL-1β-Interleukin 1 β, MPP⁺-1-methyl-4-phenylpyridinium, HPTLC–High performance thin layer chromatography, DMEM–Dulbecco's modified eagles medium, IC_{50}-Inhibitory concentration 50, DMSO–Dimethyl sulfoxide, ECL-Enhanced chemiluminescence, SDS–Sodium dodecyl sulphate, SEM–Standard error of the mean.

CONFLICT OF INTERESTS

Authors declare no conflict of interest.

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