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

MAHADEVAN M. V.1, RAMASAMY R. S.2*, BANUMATHI V.3

1Sirappu Maruthuvam Department, National Institute of Siddha, Chennai 600047, India, 2Director General, Central Council for Research in Siddha, Ministry of AYUSH, Govt of India, Govt Anna Hospital campus, Arumbakkam, Chennai 600106, India, 3Director, National Institute of Siddha, Chennai 600047, India

Email: dr.rsramaswamy@gmail.com

ABSTRACT

Objective: Parkinson's disease (PD) is one of the most common neurodegenerative disorder, It's decreased the dopaminergic neurones, tyrosine hydroxylase (TH) and increased the α-synuclein protein level. The 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-SYSY human neuroblastoma cell lines.

Methods: Mimosa pudica were pre-treated with various concentration for cell viability assay. Vehicle alone or Mimosa pudica (300µg/ml) 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 a synuclein (p<0.01) in MPP+ intoxicated cell lines.

Conclusion: The present study showed that Mimosa pudica exerts neuroprotection by suppressing a synuclein and the dopaminergic neurodegeneration. Mimosa pudica may be due to quercetin 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-SYSY, MPP+, α synuclein, Tyrosine hydroxylase, Dopamine Transporter

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 neurodegenerative disorders characterized by paucity and slowness of the movement (bradykinnesia), 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 impairment 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 Vatham 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 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 vaatha 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 vaatha 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 hypotonic action which shows its ability to penetrate the blood-brain barrier. Mimosa pudica relives “Odu vaatha” 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 SH-SYSY human neuroblastoma cell lines. The study reveals that Mimosa pudica have the abilities to improve TH and DAT proteins expression against MPP+ induced neurotoxicity, 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). SH-SYSY human neuroblastoma was procured from NCCS, Pune, india. MPP+odide, 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-SYSY 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% CO₂ 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
SH-SYSY 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 MT 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:

\[
\%\text{ Growth inhibitory rate} = \left(\frac{\text{Control OD–Test OD}}{\text{Control OD}}\right)\times 100
\]

Western blot analysis
SH-SYSY cells were seeded in 6 well poly-D-lysine precoated plates (25 μg/ml) at a density of 1X10⁶ cells/200μl/well for 24 h. Cells were treated with Mimosa pudica (1-1000μg/ml), and incubated at 37 °C for next 24 h. 20 h following mimosa treatment, cells were incubated with 5 mg/ml MT 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:

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\]

RESULTS
The present study demonstrated the neuroprotective effect of Mimosa pudica against MPP+-induced neurotoxicity in SH-SYSY cell lines.

Standardisation of Mimosa pudica
Basic phychochemical 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 15.7±1.92, 25.6±0.49 and 93.32±5.73, respectively. Quercetin content in methanolic extract of Mimosa pudica was found to be 0.20±0.30% w/w. Chromatogram of standard quercetin and Mimosa pudica were shown in fig. 2.

Cell viability assay
IC₅₀ 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. IC₅₀ value of Mimosa pudica was 211.05±3.65 in the tested conditions (fig. 1A). The higher IC₅₀ value indicates the non-toxic nature of Mimosa pudica to SH-SYSY 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 SH-SYSY cell lines.

DISCUSSION
Parkinson’s disease is a debilitating and progressive neurodegenerative 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 the 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 α-synuclein 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±0.30% 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**

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<tr>
<th>Drug Treatment</th>
<th>Control</th>
<th>MPP⁺</th>
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<tr>
<td><em>Mimosa pudica</em> 100 mg+MPP⁺</td>
<td><img src="image1" alt="Control" /></td>
<td><img src="image2" alt="MPP⁺" /></td>
</tr>
<tr>
<td><em>Mimosa pudica</em> 300 mg+MPP⁺</td>
<td><img src="image3" alt="Control" /></td>
<td><img src="image4" alt="MPP⁺" /></td>
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**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: Protein expression (A) Tyrosine hydroxylase (B) Dopamine transporter (C) α-synuclein

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
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


CONFLICT OF INTERESTS

Authors declare no conflict of interest.

REFERENCES


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