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IN VITRO EVALUATION OF THE NEUROPROTECTIVE ACTIVITY OF MOOKIRATAI ILAI KARPAM

Dr. P. Lavanya[1*], Dr. S. S. Selvapriya, [2]Dr. S. M. Chitra[3]

[1]Pg Scholar, Department of Siddhar Yoga Maruthuvam, Government Siddha Medical College, Chennai, Tamilnadu, India.

[2]Pg Scholar, Department of Siddhar Yoga Maruthuvam, Government Siddha Medical College, Chennai, Tamilnadu, India.

[3]Head of Department, Department of Siddhar Yoga Maruthuvam, Government Siddha Medical College, Chennai, Tamilnadu, India.

***Corresponding Author: Dr. P. Lavanya**

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ABSTRACT

Introduction: Kayakarpam therapies are known for rejuvenation, delaying ageing and improving vitality. Boerhavia diffusa (Mookirattai) is a well-known Siddha drug indicated mainly for Vatha disorders, which are often associated with neurological dysfunctions. However, experimental evidence for its neuroprotective effect is limited. Therefore, the present study evaluates its activity using an in-vitro model. **Aim:** To evaluate the neuroprotective potential of Mookirattai Ilai Karpam through in-vitro acetylcholinesterase inhibition. **Methods:** The formulation was prepared following classical Siddha procedures. Neuroprotective activity was assessed by an in-vitro AChE inhibition assay at different concentrations, and the percentage inhibition was compared with a standard drug. **Results:** Mookirattai Ilai Karpam showed dose-dependent inhibition of AChE. Higher concentrations exhibited notable activity, suggesting possible enhancement of cholinergic transmission. **Conclusion:** The study demonstrates that Mookirattai Ilai Karpam has significant acetylcholinesterase inhibitory activity, supporting its traditional use in Vatha-related neurological conditions. Further in-vivo and clinical validation is needed.

KEYWORDS: Siddha medicine, Mookiratai Ilai Karpam, AChE inhibition assay, neuroprotective, vadha disease.

INTRODUCTION

India is renowned for its traditional system of medicine and played a vital role in providing healthcare to the people for centuries.[1]

Siddha system of medicine is one of the most ancient traditional medical systems of India, rooted in the wisdom of the Siddhars, who emphasized preventive, promotive and curative aspects of health. According to Siddha philosophy, neurological functions are governed by the balance of Vatham, which controls sensory, motor and cognitive activities of the body. Derangement of Vatham leads to various Vatha diseases, including neurodegenerative and neurofunctional disorders.[2]

Neurodegenerative diseases are characterized by progressive neuronal damage due to oxidative stress, inflammation, excitotoxicity and apoptosis. Hence, there is a growing need for safer and effective neuroprotective agents from traditional medicine.

Mookirattai (Boerhavia diffusa Linn.) is a well-known medicinal plant widely used for anti-inflammatory, antioxidant and rejuvenative (Kayakarpam) properties[3,4] Mookirattai Ilai Karpam, a classical Siddha preparation, is traditionally indicated for strengthening the nervous system, improving vitality and restoring deranged humoral balance.[6,8]

Based on classical Siddha literature and pharmacological evidence, Mookirattai Ilai Karpam is expected to protect

neuronal cells by reducing oxidative damage, stabilizing Vatha dosham and enhancing neuronal regeneration.[4,5] Hence, the present study is undertaken to scientifically evaluate the neuroprotective activity of Mookirattai Ilai Karpam.

AIM AND OBJECTIVE

The aim of the study is to evaluate the Neuroprotective activity of Mookiratai Ilai Karpam by assessing its acetylcholinesterase inhibition through an in-vitro assay.

MATERIALS AND METHODS

STUDY DRUG SELECTION AND RATIONALE

Mookirattai Ilai Karpam, a classical Siddha herbal drug mentioned in Theraiyar Kaapiyam, was chosen for its traditional rejuvenative claim and possible neuroprotective effect.[7]

INGREDIENT

Mookiratai Ilai Karpam (Boerhavia diffusa) - 40gms.

RAW DRUG COLLECTION AND AUTHENTICATION

The raw drug was collected from ponneri village, Thirpattur-Dt, Tamil Nadu. The study drug was identified and authenticated by the pharmacologist of Siddha Central Research Institute (Central Council For Research In Siddha, Chennai, Ministry of Ayush, Government of India), Aringar Anna Government Hospital for Indian Medicine and Homeopathy Campus, Arumbakkam, Chennai-106.

PURIFICATION OF HERB

It was thoroughly cleaned and given a good rinse with fresh water following collection. It was then allowed to dry completely in the shade for two weeks.

PREPARATION OF MOOKIRATTAI ILAI KARPAM

The cleaned and dried Mookirattai leaves was powdered using a grinding machine. The material was ground into a fine powder and filtered using the cotton cloth (Vasthirakayam method).

Boerhavia diffusa (plant) **Dried Leaves** **Dry Powder**

Figure 1: Preparation of Mookirattai Ilai Karpam.

IN VITRO NEUROPROTECTIVE ACTIVITY

In-Vitro AChE enzyme Inhibition Assay

AChE activity was measured using a modified 96-well microplate assay based on Ellman's method, enzyme hydrolyses the substrate acetylthiocholine resulting in the product thiocholine which reacts with Ellman's reagent (DTNB) to produce 2-nitrobenzoate-5-mercaptothiocholine and 5-thio-2-nitrobenzoate which can be detected at 412 nm. 50 mM Tris–HCl pH 8.0 was used as a buffer throughout the experiment. AChE enzyme stock solution (518 U/ml) was kept at -80oC and the enzyme-dilution was done in 0.1% BSA in buffer. DTNB was dissolved in the buffer containing 0.1 M NaCl and 0.02 M MgCl2. ATCI was dissolved in deionized water. In the 96-well plates, 100 µl of 3 mM DTNB, 20 µl of 0.26 U/ml of AChE, and 40 µl of buffer (50 mM tris pH 8.0), to which 20 µl of test drug in various concentrations (25, 50, 100, 250 and 500 µg/ml in water) dissolved were added to the wells. After mixing, the plate was incubated for 15 min (25oC).The enzymatic reaction was initiated by the addition of 20 µl of 15 mM acetylthiocholine iodide and the hydrolysis of acetylthiocholine was monitored by reading the absorbance every 5 min for 20 min at 412 nm. Physostigmine (5, 10, 20 and 40 µg/ml) was used as positive control. All the reactions were performed in triplicate.[9,10,11]

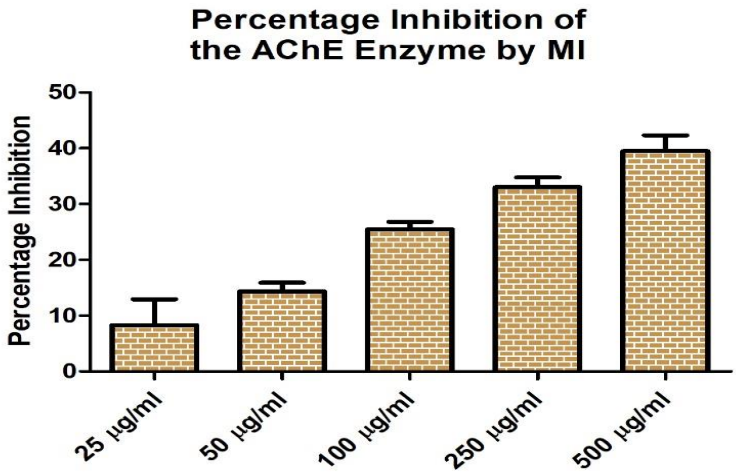
RESULTS

Results are expressed as Mean ± SD.

Percentage Inhibition of AChE Enzyme by Test Drug - MI

Concentration of MI in µg/ml	Percentage Inhibition of AChE Enzyme by Test Drug
MI 25	8.321 ± 4.607
MI 50	14.34 ± 1.546
MI 100	25.48 ± 1.312
MI 250	33.02 ± 1.769
MI 500	39.49 ± 2.823

Each value represents the mean ± SD. N=3



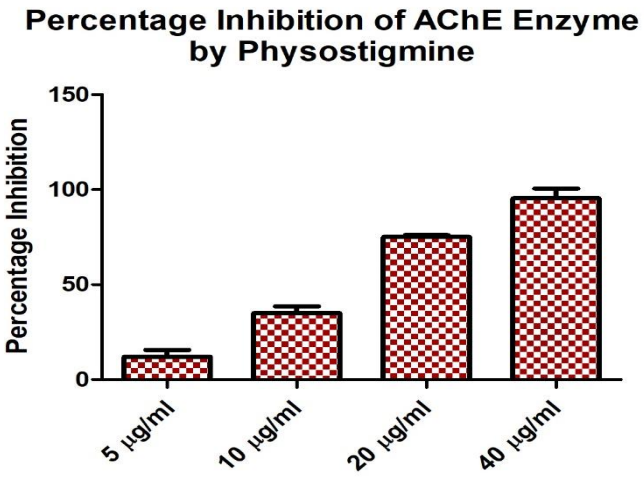
Percentage Inhibition of AChE Enzyme by Standard Drug

Concentration of Physostigmine in µg/ml	Percentage Inhibition of AChE Enzyme by Std Drug
5	11.98 ± 3.605
10	34.92 ± 3.687
20	75.14 ± 0.9114
40	95.49 ± 5.104

Each value represents the mean ± SD. N=3

IC 50 Value of test and Standard drug

IC 50 Value of test drug MI	679.7 ± 86.54
IC 50 Value of Std drug Physostigmine	16.84 ± 0.643



The result obtained from the present clearly indicates that the test drug MI was effective in inhibiting AChE enzyme at stipulated concentration dose dependently. Maximum percentage inhibition of about 39.49 ± 2.823 % was observed at 500 µg/ml with the IC 50 value of 679.7± 86.54 µg/ml when compare to that of the Physostigmine, a known AChE Inhibitor with the maximum inhibition 95.49± 5.104 % at the concentration of 40 µg/ml with the IC 50 value of 16.84±0.643 µg/ml.

DISCUSSION

Inhibition of acetylcholinesterase (AChE) is an important therapeutic approach in the management of neurodegenerative disorders. The present study demonstrated that the test drug MI exhibited a concentration-dependent inhibitory effect on AChE activity. A maximum inhibition of 39.49 ± 2.823% was observed at 500 µg/ml, with an IC_{50} value of 679.7 ± 86.54 µg/ml, indicating moderate enzyme inhibitory potential. Compared to physostigmine, the standard AChE inhibitor, MI showed lower inhibitory efficacy. Physostigmine produced 95.49 ± 5.104% inhibition at 40

µg/ml with an IC_{50} value of 16.84 ± 0.643 µg/ml, highlighting its superior potency. The comparatively mild activity of MI may be attributed to the presence of multiple phytoconstituents acting synergistically rather than a single potent inhibitor. Despite its lower potency, the dose-dependent AChE inhibition suggests that MI may contribute to neuroprotective effects. Further studies involving phytochemical characterization and in vivo evaluation are required to substantiate its therapeutic potential.

CONCLUSION

The In-Vitro AChE enzyme Inhibition Assay results indicate that the siddha formulation Mookiratai Ilai karpam shows promising neuroprotective activity in the estimated AchE enzyme Inhibition Assay. The protective effect of Mookiratai ilai Karpam may be due to antioxidant and anti-inflammatory properties, helping to reduce neuronal damage and balance vatha kutram which aligns with the action of Karpam Marunthu as mentioned in classical Siddha text. These results validate the accuracy of Siddha texts based on scientific parameters. Further pre-clinical and clinical studies are needed to evaluate the effectiveness and provide strong evidence of Siddhar's science in the future.

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Nil.

Conflicts of interest

None

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