In Vitro Inhibition Assessment of Acetyl Cholinesterase by Mahabhutarava Ghrta

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ABSTRACT

Psychiatric disorders can make a huge negative impact not only to the patient but also to the family and his society. Schizophrenia is one among them that may lead to alteration in cognition and conation. Ayurveda considers the usage of ghrta preparations as the best strategy for the management of such psychiatric ailments. In the present study, a ghrta yoga, Mahabhutaravaghrta which had been in practise for the management of Schizophrenia and other psychiatric conditions was selected and an invitro inhibition assessment of Acetylcholinesterase has been planned. If inhibition is identified in the ghrta, it can justify the clinical efficacy of the drug in the patients.

Keywords: -Mahabhutaravaghrta, Acetylcholinesterase, Schizophrenia, Unmada

INTRODUCTION

Schizophrenia is one of the worst diseases affecting the mankind, which is puzzling the society for long¹. Treatment options in modern medicine include drug treatment, electroconvulsive therapy and psychosocial rehabilitation methods². Tranquilizers, barbiturates, antipsychotics etc. are used for drug therapy. Among them antipsychotics are the foremost choice³. 70% of patients treated with any antipsychotic achieve remission⁴. But certain adverse effects including renal diseases, dry mouth, constipation, blurred vision, memory loss, weight gain, diabetes etc. are reported as well⁵. The search for safer as well as effective therapeutics add to the scope for newer interventions through Ayurveda.

In Ayurveda, the broad spectrum of psychiatric disorders are discussed under Unmada. Various herbal and herbo-mineral preparations explained in classics for the management of unmada. Among them, ghrta play an important role in the treatment of unmada. The most highlighted property of ghrta is that it assimilates the property of drugs added to it providing a synergetic action in combination⁶. Blood brain barrier has a lipophilic structure. Lipids and lipid soluble drugs can pass easily through blood brain barrier⁷. Ghrta imparts a lipid medium. This may help in the fast absorption of ghrta formulations to the target areas of central nervous system. Mahābhūtarāvaghṛta is a formulation mentioned under Bhūtapṛatiṣedhaadhyāya in Aṣṭāṅga Hṛdaya Uttara sthāṇa⁸. Main indications are Unmāda and graha⁹. It is being used in clinical practice in conditions of Unmāda.

Acetylcholine is a neurotransmitter at neuromuscular junctions. Acetylcholine receptors in the central nervous system represents a new therapeutic target in many psychiatric diseases. The role of Acetylcholinesterase⁰ inhibitors in the management of Schizophrenia is accepted. The effects of cognitive impairment on the occupational functioning, social activity and economic life of patients with Schizophrenia constitute major obstacles to recovery. It has been reported that both nicotinic and muscarinic receptors play crucial roles in cognition and they may be
considered potential therapeutic targets for new drugs designed to decrease cognitive deficits. So a study has been planned to assess the in vitro inhibition assessment of Acetylcholinesterase by Mahābhūtarāvaghṛta.

**In vitro study**

**Assessment** - Ellman Spectrophotometric Method

**Setting** - Research & Development Department Aryavaidyasala, Kottakkal

**Observations on In-vitro study**

<table>
<thead>
<tr>
<th>S.N</th>
<th>Sample</th>
<th>Dose</th>
<th>Absorbance at 412 nm</th>
<th>% Enzyme Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>01</td>
<td>Sample without drug (Q)</td>
<td></td>
<td>0.756 AU</td>
<td></td>
</tr>
<tr>
<td>02</td>
<td>Sample with Methanol extract of Gṛta</td>
<td>10ml</td>
<td>0.734 AU</td>
<td>32.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20ml</td>
<td>0.640 AU</td>
<td>45.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30ml</td>
<td>0.543 AU</td>
<td>57.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>40ml</td>
<td>0.449 AU</td>
<td>70.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50ml</td>
<td>0.352 AU</td>
<td>83.0</td>
</tr>
<tr>
<td>03</td>
<td>Positive control, Eserine</td>
<td>0.4 g</td>
<td>0.339 AU</td>
<td>85.0</td>
</tr>
</tbody>
</table>

The above table gives the absorbance at 412 nm in the sample drug, methanol extract of gṛta and also in the positive control Eserine drug.

**Discussion on in-Vitro study**

**Introduction**

Acetylcholinesterase (AchE) is an enzyme that catalyses the breakdown of acetylcholine (Ach) that function as neurotransmitter. AchE catalyses the hydrolysis of acetylcholine to acetate ion and choline. During neurotransmission, Ach is released from the presynaptic neuron in to the synaptic cleft and binds to Ach receptors on the postsynaptic membrane. AchE also located on the postsynaptic membrane terminates the signal transmission by hydrolyzing Ach. Acetylcholinesterase inhibitor inhibit Acetylcholinesterase from breaking down acetylcholine in to choline and acetate, thereby increasing both the level and duration of action of neurotransmitter acetylcholine in the CNS. The Acetylcholinesterase enzyme is the target for a drug / formulation which play its role in the prevention of hydrolysis of the neurotransmitter acetylcholine. Such inhibitors are the most effective approach to treat the cognitive symptoms of neurodegenerative diseases.

**Method - Ellman Spectrophotometric Method**

**Principle** - Neurodegeneration by Acetyl cholinesterase

**Methodology**

Acetyl cholinesterase (AChE) hydrolyses the Acetyl choline to produce thiocholine, which in turn reacts with Dithiobisnitrobenzoate (DTNB) to produce a yellow 5-thio-2-nitrobenzoic acid (TNB). The colour intensity of the product is measured at 412 nm and it is proportional to the enzyme activity.

**Ellman Method**

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\[ \text{AchE inhibition activity (\%)} = 1 - \frac{S \times 100}{Q} \]

Where, \(S=\)Absorbance at 412 nm with methanol extract of gṛta

\(Q=\)Absorbance at 412 nm as the 100 % initial activity with methanol extract of plain gṛta
The concentration of the trial drug, where the enzyme inhibition was reduced to half of the full activity sample (Q), (the reaction mixture without drug), that concentration of the trial drug is called IC 50 concentration. If this absorbance (S) is low or comes nearer to that of full activity of positive control, the drug can be considered as a good AChE inhibitor. A therapeutic agent having low IC 50 is said to have good Enzyme inhibitory property.

**Eserine (Positive control)**

Physostigmine (Eserine) is a reversible cholinesterase inhibitor that enhances the transmission of acetylcholine signals in the brain and can cross blood brain barrier. In clinical trials, it was not shown to confer convincing benefits and it led to side effects like nausea, vomiting, diarrhoea, loss of appetite etc. resulting in a high rate of withdrawal.

**Pharmacology**

Physostigmine acts by interfering with the metabolism of acetylcholine. It is a covalent inhibitor of acetyl cholinesterase, the enzyme responsible for the breakdown of acetylcholine in the synaptic cleft of neuromuscular junction. It indirectly stimulates both nicotinic and muscarinic acetylcholine receptors.

**Bioactivity**

Physostigmine functions as an acetyl cholinesterase inhibitor. Its mechanism is to prevent the hydrolysis of Ach by AchE at the transmitted sites of Ach. This inhibition enhances the effect of Ach, making it useful for the treatment of cholinergic disorders.

**Inference**

The ghṛta is giving above 50% AChE inhibition at the concentration of extract of 30 ml. 50 ml of the ghṛta (Methanol extract) is giving similar enzyme inhibition equivalent of Positive Control. From the above results, it is inferred that 50 ml ghṛta may have equivalent therapeutic efficacy of 0.4 g Eserine drug. This shows that minimum 50 ml ghṛta should be given to a patient to get the symptomatic improvement.

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**CONCLUSION**

Acetylcholinesterase inhibitors proved to be effective in the management of psychiatric diseases such as Schizophrenia. Invitro inhibition assessment studies conducted in Mahābhūtarāvaghṛta, proved that AChE inhibitionoccurs at the concentration of extract of 30 ml. Thus the study drug can be a promising combination in the management of Schizophrenia and future researches can be planned in many other psychiatric diseases.

**REFERENCES**