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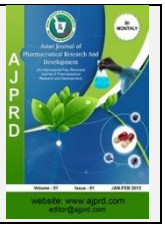

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Review Article

In Vitro Inhibition Assessment of Acetyl Cholinesterase by Mahabhutarava Ghrta

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A B S T R A C T

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

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INTRODUCTION

Schizophrenia is one of the worst diseases affecting the mankind, which is puzzling the society for long[1].Treatment options in modern medicine include drug treatment, electroconvulsive therapy and psychosocial rehabilitation methods[2]. Tranquilizers, barbiturates, antipsychotics etc. are used for drug therapy. Among them antipsychotics are the foremost choice[3]. 70% of patients treated with any antipsychotic achieve remission[4]. But certain adverse effects including renal diseases, dry mouth, constipation, blurred vision, memory loss, weight gain, diabetes etc. are reported as well[5]. 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 combination[6]. Blood brain barrier has a lipophilic structure. Lipids and lipid soluble drugs can pass easily through blood brain barrier[7]. 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 *Aṣtāṅga Hṛdaya Uttara sthāna*[8]. Main indications are *Unmāda and graha*[8].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 Acetylcholinesterase[9] 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[9]. 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

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-nitrobenzoic acid (TNB). The colour intensity of the product is measured at 412 nm and it is proportional to the enzyme activity.

Observations on In-vitro study

Table 01: Comparison of Enzyme inhibition Absorbance of different sample dilutions and Positive control

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

The above table gives the absorbance at 412 nm in the sample drug, methanol extract of ghrta 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 post synaptic 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 - Neuro degeneration by Acetylcholinesterase

$(CH_3)3N^+CH_2CH_2O\text{-}CO\text{-}CH_3 + H_2O \rightarrow (CH_3)3N^+CH_2CH_2OH + CH_3COOH$

Acetyl Choline AChE Choline Acetic acid

Ellman Method

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. Electric eel AchE was used for assay, the reaction mixture contained 0.02 ml AchE (19.93 unit/ml buffer, pH 8), X ml *Ghṛta* Methanol extract (mentioned in the table), 1ml buffer, 0.01 ml. 0.5mM DTNB (2-nitro benzoic acid) and 0.02 ml. 0.6mM Acetylthiocholine iodide solution. The reaction mixture was incubated at 37 deg C for 20 min. The yellow anion produced by the reaction of thiocholine from the reaction with DTNB in the reaction mixture measured at 412 nm. Eserine was used as the positive control. The more absorbance at 412 nm indicates more enzyme activity, and which reduced by the drug, indicates the inhibition.

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

Where, S =Absorbance at 412 nm with methanol extract of *ghṛta*

Q = Absorbance at 412 nm as the 100 % initial activity with methanol extract of plain *ghṛta*

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.

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