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Original Article

Acetylcholinesterase activity of Ocimum Sanctum leaf extract

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ABSTRACT

Introduction: Ocimum sanctum leaf being an herb for all reasons, is multifunctional and a highly used herb for curing many ailments. Acetylcholinesterase is an enzyme produced in our bodies that is a primary cholinesterase and is mainly found in the neuromuscular junctions and junctions of chemical synapses of the cholinergic type. Its primary activity in such sites is to inhibit/terminate synaptic transmission. This study was performed so as to observe and evaluate the acetylcholinesterase activity of Ocimum sanctum leaf at various concentrations. On performing this study, we will be able to evaluate how potent Ocimum sanctum leaf extract is in inhibiting the enzyme Acetylcholinesterase. Materials and Methods: Ocimum sanctum extract was examined for their AchE inhibitory by the spectrophotometric method developed by Ellman et al, activities at different concentrations (5, 10, 20, 40, 80, 160 and 320 micrograms/ml) and were dissolved in a base- tris (0.05 M) buffer, following the spectrophotometric method. Results: Different concentrations of Ocimum sanctum extract were evaluated for the inhibitory effect on the activity of acetylcholinesterase. The plant extract exhibited potent inhibition of the ACh. The IC50 was found to be 54.57µg/ml. Maximum inhibition was found to be 83.16% at 320µg/ml. Conclusions: On performing the in vitro study, it was found that the ethanolic extract of Ocimum sanctum was a potent inhibitor of acetycholinesterase and therefore acts as an anticholinergic agent. Thus, giving scope for more revolutionary breakthroughs in the field of pharmacological actions of various plant extracts.

Keywords: Tulsi, acetylcholinesterase, memory, stimulant, ocimum sanctum.

Introduction

Tulsi, (Ocimum sanctum), is a multifunctional medicinal herb used in the indigenous system of medicine. Ocimum sanctum has been adored in almost all ancient Ayurvedic texts for its extraordinary medicinal properties. It is pungent and bitter in taste and hot, light and dry in effect. Its seeds are considered to be cold in effect [1].

The roots, leaves and seeds of Ocimum sanctum possess several medicinal properties. Ayurvedic texts categorize Ocimum sanctum as a stimulant, aromatic and antipyretic [2-4]. It has a wide range of action on the human body mainly as a cough alleviator, a sweat-inducer and a mitigator of indigestion and anorexia. Ocimum sanctum has been considered a multifunctional herb, that works many wonders and has therefore been used as a cure for most diseases.

Ocimum sanctum has a variety of biological or pharmacological activities such as antibacterial, antiviral, anti-fungal, antiprotozoal, antimalarial, anthelmintic, antidiarrhoeal, analgesic, antipyretic, anti-inflammatory, antiallergic, antihypertensive, cardioprotective, central nervous system (CNS) depressant, memory enhancer, antihypercholesterolaemic, hepatoprotective, anti-diabetic, anti-asthmatic, antithyroidic, antioxidant, anticancer, chemopreventive, radioprotective, immunomodulatory, anti-fertility, anti-ulcer, anti-arthritic, adaptogenic / anti-stress, anti-cataract, antileucodermal and anticoagulant activities [1,5,6].

As stated above, one of the major functions of Ocimum sanctum or Tulsi is its memory enhancing effect. A dried whole plant of Ocimum sanctum ameliorated the amnesic effect of scopolamine [1,7,8].

Ethanolic Ocimum sanctum leaf extract increased step-down latency (SDL) and acetylcholinesterase inhibition significantly. Ethanol is used to elute Ocimum sanctum leaf extract because of its increased polarity, therefore increasing its ability to dilute polar and non-polar extracts. Hence, it can be employed in the

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treatment of cognitive disorders such as dementia and Alzheimer's disease [2-4].

Acetylcholinesterase is an enzyme produced in our bodies that is a primary cholinesterase and is mainly found in the neuromuscular junctions and junctions of chemical synapses of the cholinergic type. Its primary activity in such sites is to inhibit/terminate synaptic transmission [5, 6,9,10].

In cases of Dementia and Alzheimer's, acetylcholinesterase increase results in the impairment of cognitive functioning of the brain due to an abnormality in brain cholinergic activity [7,11]. Therefore, in such cases Ocimum sanctum, can be used in increasing the acetylcholinesterase inhibition, which will further result in restoring the senile, cognitive dysfunctioning of Dementia and Alzheimer's patients [2, 8, 9].

As the world progresses and we as humans continue to evolve faster than how the earth spins around its own axis, it becomes pivotal for us to develop less invasive, less chemical drugs which will help in curing disorders such as dementia and Alzheimer's [9, 10].

The objective of this study was observe and evaluate the acetylcholinesterase activity of tulsi leaf extract at various concentrations, Ocimum sanctum being a wide spread herb available to the common public.

On performing this study, there might be a new shed light on how ethanolic extract of Ocimum sanctum leaf might act as a stimulant for maintaining the functionality of neurotransmitters and restoring cognitive functioning.

Materials and Methods

In vitro acetylcholinesterase inhibition assay

This in vitro study was conducted by using ethanolic extract of Ocimum sanctum leaf extract which was obtained from Green Chem, Herbal Extracts and Formulations, Bengaluru.

Ethanolic extract of Ocimum sanctum extract was examined for their AchE inhibitory activities at different concentrations (5,10,20,40,80,160 and 320 micrograms/ml) and were dissolved in a base- tris (0.05 M) buffer, following the spectrophotometric method developed by Ellman et al. [6] (1961) as described by Salles et al. (2003) [11]. In this method, 200 microlitre of acetylthiocholine iodide (15mM), 100 microlitre of DTNB (3mM) and 200 microlitre of test extract solution at the different concentrations were mixed and incubated for 15 mins at 30 degrees Celsius. Then the mixture was monitored spectrophotometrically at 412 mm ten times, each 13s. After that 200 microlitre of AChE (0.3U/ml) solution were added to initial mixture, to start the reaction and then the absorbance was determined.

Control contained all components except the tested extracts. The percentage of AchE inhibitory activity (%IA) was calculated by using the following equation:

IA (%)= (Activity of Control -Activity of Tested)/Activity of Control x100

All treatments were performed in triplicate with two replicates.

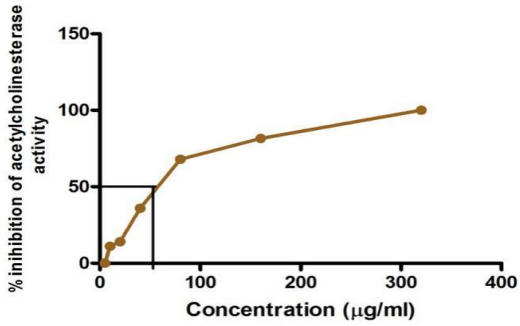
Examination IC_{50} values

The concentrations of the tested extracts that inhibited the hydrolysis of substrate (acetylcholine) by 50% (IC_{50}) were determined by a liner regression analysis between the inhibition percentages against extract concentrations by using the graph pad prism, depicted in Figure 1.

Results

Different concentrations of Ocimum sanctum extract were evaluated for the inhibitory effect on the activity of acetylcholinesterase. As the liner progression depicted below in Figure 1 between percentage inhibition against different concentrations of ethanolic extract of Ocimum sanctum extract exhibited potent inhibition of the ACh. The IC_{50} was found to be 54.57µg/ml. Maximum inhibition was found to be 83.16% at 320µg/ml.

Figure 1. Graph pad prism analysis



Discussion

The extract used for this study was an ethanolic extract of Ocimum sanctum leaf. Ocimum sanctum also known as a multifunctional herb performs a wide spectrum of roles such as an anticonvolusant, antimalarial, and an active acetylcholinesterase inhibitor [1, 2, 6, 7, 11]. Ocimum sanctum extract, on performing various phytochemical analysis was found to break down the enzyme acetylcholinesterase, and is therefore considered an anti-acetylcholinesterase [12,13]. This results in the assimilation of the Acetylcholine by the brain and therefore promotes the cognitive functioning of the brain, REM sleep, and enhances memory [9, 14-16]. In this study, the acetylcholinesterase inhibition activity of the ethanolic extract of Ocimum sanctum leaf was analyzed in various concentrations and the IC_{50} value was found to be 54.57µg/ml. Therefore, proving that Ocimum sanctum truly is a potent inhibitor of AChE.

Acetylcholinesterase, also known as AChE or acetylhydrolase, is the primary cholinesterase in the body. It is an enzyme that catalyzes the breakdown of acetylcholine and of some other choline esters that function as neurotransmitters [17,18]. AChE is found at mainly neuromuscular junctions and in chemical synapses of the cholinergic type, where its activity serves to terminate synaptic transmission [9,16]. It belongs to carboxylesterase family of enzymes. It is the primary target of inhibition by organophosphorus compounds such as nerve agents and pesticides.

In a study performed by H. Joshi, it was observed that Ocimum tenuiflorum increased SDL in both young and aged mice when subjected to passive avoidance paradigm, indicating its potent anti-amnesic activity. The central cholinergic system plays an important role in learning and memory. Phenytoin is known to reduce hippocampal ACh concentration. In their study, they used phenytoin per se (12 mg/kg, p.o.) which significantly elevated brain AChE activity, whereas piracetam (250 mg/kg, p.o.) and O. tenuiflorum (50, 100, and 200

mg/kg, p.o.) lowered this activity significantly ($P < 0.05$). Hence O. tenuiflorum may be useful as a nootropic agent in the early management of various cognitive disorders [19].

Similarly, many studies were performed with other herbal extracts regarding their acetylcholinesterase inhibiting activity. A study performed by Lakshmi et al., to evaluate the acetyl cholinesterase inhibitory activity of Acacia catechu ethanolic seed extract to introduce a new source for management of Alzheimer's disease [4].

As observed most of the herbal extracts have a potent acetylcholinesterase inhibiting potential, another study performed by Vijayasree Vayalanellore Giridharan et al., on extracts of Ocimum sanctum Linn. Leaves proved that they act as an antidementic and anti-cholinesterase agent and also as an immunostimulant in rats. Acetylcholinesterase (AChE) activity was estimated in different parts of the brain, and immune status was studied using dinitrochlorobenzene (DNCB) skin sensitivity tests. In all the three models, both aqueous and alcoholic Ocimum sanctum extracts decreased the time taken to reach the shock-free zone and the number of mistakes and significantly decreased the AChE activity in rats. Ocimum sanctum treatment significantly increased the induration in the DNCB skin test. Therefore, Ocimum sanctum was shown to be useful for the management of experimentally induced cognitive dysfunctions in rats [20].

Therefore, substantiating that Ocimum sanctum extracts alcoholic or aqueous possess potent acetylcholinesterase inhibition activity.

In this study, ethanolic extract was preferred due to its increased polarity, therefore having an increased efficacy to elute polar and non-polar plant extracts. On taking various concentrations of Ocimum sanctum leaf extract (5,10,20,40,80,160 and 320 micrograms/ml), and subjecting it to an in vitro assay by the spectrophotometric method developed by Ellman et al. [6], it was observed that Ocimum sanctum had a substantial inhibitory potential against Acetylcholinesterase. The linear regression analysis between percentage inhibition of acetylcholinesterase against extract concentrations was done by using the graph pad prism. On analyzing the graph pad prism in Figure 1, the IC_{50} was found to be 54.57µg/ml and the maximum inhibition was found to be 83.16% at 320µg/ml. Thereby, indicating that Ocimum sanctum leaf extract showed maximum inhibitory potential at the concentration of 320µg/ml. This study therefore reinstates that Ocimum sanctum truly is an herb of many wonders.

Conclusion

On performing the in vitro assay with various concentrations of ethanolic extract of Ocimum sanctum leaf, (5,10,20,40,80,160 and 320 micrograms/ml), using the spectrophotometric method developed by Ellman et al. [6]. The IC_{50} value was found to be 54.57µg/ml. Maximum inhibition was found to be 83.16% at 320µg/ml, which is depicted in Figure 1. Therefore, it is observed that Ocimum sanctum is a potent inhibitor of Acetylcholinesterase and has the efficacy to act as an anticholinergic agent, thus personifying the multifunctional abilities of Ocimum sanctum. Thus giving scope for more revolutionary breakthroughs in the field of pharmacological actions of various plant extracts.

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