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IN-VITRO INHIBITION OF ACETYLCHOLINESTERASE BY *CITRULLUS VULGARIS* SEED EXTRACT: POSSIBLE ROLE IN MEMORY ENHANCEMENT

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ABSTRACT

Alzheimer's disease is a form of dementia characterized by loss of central cholinergic neurons associated with a marked reduction in content of acetyl cholinesterase. Acetyl cholinesterase inhibitors are used clinically for the treatment of mild to moderate neurodegenerative diseases such as Alzheimer's disease. In the present study an attempt has been made to explore the Acetyl-cholinesterase inhibitory potential of *Citrullus vulgaris* seeds which has not been scientifically documented. Seeds of *Citrullus vulgaris were extracted* by using n-hexane solvent. Acetyl cholinesterase activity was measured using a UV spectrophotometry by Ellman's method in the presence or absence of the extracts. Galanthamine was used as a positive control. The extract of *Citrullus vulgaris* showed more than 50% AChE inhibitory activity. The concentration required for 50% enzyme inhibition (IC_{50} value) was found to be 57.54µg/ml. Thus, *C. vulgaris extract* acts as a potent inhibitor of AChE which might be useful in improving memory and other cognitive functions associated with the cholinergic system.

Keywords: Alzheimer's disease, Acetyl-cholinesterase, seeds.

INTRODUCTION

The brain is the central part of our body that controls physiological and cognitive functions. Normal brain functioning is disturbed due to loss or damage to neurons leading to loss of memory and cognition. Cholinergic system plays an important role in memory and cognition. Decrease in acetylcholine level due to damage to central cholinergic neurons is thought to be one of the factors for loss of memory. Loss of memory is the main symptom of brain damage and for a variety of disorders including Alzheimer's disease. Acetyl cholinesterase (AChE) is the main enzyme for the breakdown of acetylcholine. Acetyl cholinesterase inhibitors are used clinically used in treatment of mild to moderate neurodegenerative diseases such as Alzheimer's disease. Herbal medicines can be a new source of inhibitors of this enzyme [1].

Citrullus vulgaris (Family: cucurbitaceae) is commonly known as water melon. The watermelon fruit has deep green or yellow colored smooth thick exterior rind with gray or light green vertical stripes. Inside the fruit is pink, red or even yellow in color with small black seeds embedded in the middle third of the flesh. The ripe fruits are edible and largely used for making confectionary. Its nutritive values are also useful to the human health. Fruit is used in cooling, strengthening, aphrodisiac, astringent to the bowels, indigestible, expectorant, diuretic, and stomachic, purifies the blood, allays thirst, cures biliousness, good for sore eyes, scabies and itches and as brain tonic to the brain [2]. It also reported having analgesic and anti-inflammatory activity of roots and leaves [3], antimicrobial activity [4], laxative activity of fruit [5], anti-oxidant and antiulcerative activity [6].

In the present study an attempt has been made to explore the Acetyl-cholinesterase inhibitory potential of

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Citrullus vulgaris seeds which has not been scientifically documented.

MATERIALS AND METHODS

Chemicals

Acetylthiocholine iodide (ATCI), 5-5'-thiobis-2-nirobenzoic acid (DTNB), Tris [hydroxymethyl] amino methane (Tris HCl buffer), Galanthamine was obtained from "Dr. Reddys" used for the experiment. Acetyl cholinesterase enzyme was obtained from fresh chicken liver homogenate. TLC plate (silica gel F254 0.2mm, Aluminium sheet), chloroform, methanol, n-hexane were purchased from LOBA, Mumbai.

Extraction of Plant material

The seeds of *Citrullus vulgaris* were collected from local market of Kolhapur city, Maharashtra, India. The seeds were ground into fine powder and were extracted with 300 mL of n-hexane for 24hr using soxhlet apparatus. Extract was filtered and concentrated to dryness in a rotary evaporator.

Preliminary Phytochemical Screening

The n-hexane extract was subjected to phytochemical tests for the presence of different constituents using standard methods [7].

Acetyl cholinesterase activity assay

Microplate assay for AChE activity

Acetyl cholinesterase inhibitory activity of *C. vulgaris extract* was determined by Ellman's method. Enzyme activity was measured by the method of Ellman *et al*., [8]. Enzyme activity reaction mixture (200 µl) consisted of 160µl of 50 mM Tris HCl buffer, pH 7.4, with/without plant extract followed by the addition of 10µl enzyme (40-60 g protein) from fresh chicken liver homogenate in 96-well plates. The contents were mixed and preincubated for 10 min at 25^0C. The reaction was initiated by the addition of 10µl of 1 mM DTNB and 3 mM substrate acetylthiocholine iodide (ACTI). After 15 min incubation, absorbance was measured at 412 nm within 4-7 min. Control experiments were carried out to correct for non-enzymatic hydrolysis by adding enzyme after the addition of DTNB. Absorbance values were subtracted from the control and data presented as percent inhibition of enzyme activity. All experiments were carried out with their respective controls in triplicate [8-9].

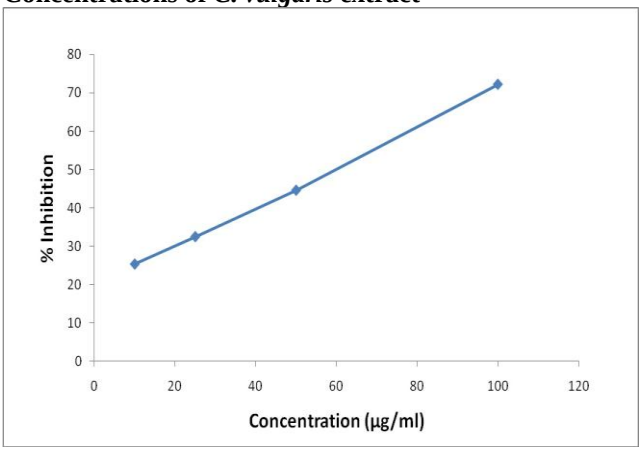
$$\text{Percentage inhibition of AchE} = \frac{[(A-B) \times 100]}{A}$$

Where, A is the change in absorbance of the assay without the plant extract and B is the change in absorbance of the assay with the plant extract

Thin layer chromatography (TLC) with bioassay detection for AChE inhibition

The TLC with bioassay detection for AChE inhibition was modified from previous studies [10-11]. TLC plate (silica gel F254 0.2mm, Aluminium sheet was used as a stationary phase. 20 microliters of extract and 10 microliters of Galanthamine (1.5mg/ml) dissolved in chloroform-methanol (8:2), were spotted on a TLC plate. Chloroform-methanol (8:2) mixture was used as the mobile phase for the development of the TLC plate. The plate was allowed to dry at room temperature, then it was sprayed with 1mM ATCI and 1mM DTNB in Tris-HCl, pH:8. After 3-5 minutes drying, the plate was sprayed with 3 Unit/ml AChE in Tris-HCl, pH: 8. 20 minutes later, a yellow background appeared; occurrence of white spots marked positive reaction. A positive spot indicating AChE inhibitor was a colorless spot on the yellow background.

Fig 1. Percentage Inhibition of AChE at different Concentrations of *C. vulgaris* extract



RESULTS AND DISCUSSION

Alzheimer's disease is a form of dementia characterized by loss of central cholinergic neurons associated with a marked reduction in content of acetyl cholinesterase (AChE), the enzyme responsible for the termination of nerve impulse transmission at cholinergic synapses. Consequently, one therapeutic approach to treatment of Alzheimer's disease is use of anti-cholinesterase drugs. The seed extract showed the presence of glycosides and fixed oils. Ellman's reaction is commonly used to screen the activity of AChE. Measurement of AChE activity is based on the yellow colour produced from thiocholine (product from acetyl thiocholine) when it reacts with dithiobisnitrobenzoate ion [8,12]. AChE is synthesised mainly in hepatocytes which will then release the enzyme into the blood which distributes it to its target sites. AChE terminates the action of acetylcholine post-synaptically. The results showed that the oil of *C. vulgaris* seeds inhibited AChE in a concentration dependent manner. The maximum inhibition (72.25%) was observed at the final assay concentration of 100 µg/mL The IC_{50} value calculated from the equation obtained from the log concentration versus inhibition curve was 57.54 µg/mL. By means of

TLC with bioassay detection for AChE inhibition, the extracts from *C. vulgaris* showed spots of AChE inhibitors. This result suggests that the AChE inhibitors can be used for the isolation of acetyl cholinesterase inhibitors, which are widely used in the treatment of Alzheimer's disease.

CONCLUSION

The present study indicated that *C. vulgaris extract* acts as a potent inhibitor of AChE which might be useful in improving memory and other cognitive functions associated with the cholinergic system.

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