



Research Article

EVALUTION OF ANTI CHOLINESTERASE ACTIVITY OF METHANOLIC LEAF EXTRACT OF *OCIMUM BASILICUM*

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Received on: 08-04-2019; Revised and Accepted on: 03-06-2019

ABSTRACT

The present study was undertaken to evaluate Anti cholinesterase activity of methanolic leaf extract of *Ocimum basilicum*. Different parts of the *Ocimum basilicum* species are used in India for a wide range of medicinal purposes. Anti cholinesterase effect of methanolic leaf extract of *Ocimum basilicum* species was studied by using frog's rectus abdominus muscle. Anti cholinesterase activity was studied on frog's rectus abdominal muscle. Methanolic leaf extract of *Ocimum basilicum* species as a test and Neostigmine is used as standard. Present study evaluated height of contraction. Acetylcholine shows significant increased in height of contraction in presence of neostigmine. The present results indicated that a significant increase in height of contraction was observed at the doses of 2mg, & 4mg of *Ocimum* extract. Compared to Neostigmine, a drug with narrow therapeutic window, *Ocimum basilicum* showed wide therapeutic window.

KEYWORDS: Anti cholinesterase; frog's rectus abdominus muscle; *Ocimum basilicum* species Myasthenia gravis.

INTRODUCTION

Cholinesterase is a family of enzymes that catalyzes the hydrolysis of the neurotransmitter acetylcholine (ACh) into choline and acetic acid, a reaction necessary to allow a cholinergic neuron to return to its resting state after activation.

Acetyl cholinesterase is found in many types of conducting tissue: nerve and muscle, central and peripheral tissues, motor and sensory fibers, and cholinergic and noncholinergic fibers. The activity of AChE is higher in motor neurons than in sensory neurons [1, 2]. The enzyme exists in multiple molecular forms, which possess similar catalytic properties, but differ in their oligomeric assembly and mode of attachment to the cell surface. In the mammalian brain the majority of AChE occurs as a tetrameric, G4 form (10) with much smaller amounts of a monomeric G1 (4S) form [3]. Pseudo cholinesterase also known as plasma cholinesterase, butyrylcholinesterase, or acylcholine acylhydrolase, is found primarily in the liver. Different from AChE, BuChE hydrolyzes butyrylcholine more quickly than ACh [4]. The liberated choline

from the ACh decomposition is taken up again by the pre-synaptic nerve and the neurotransmitter is synthesized by combining with acetyl-CoA through the action of choline acetyltransferase [5, 6].

ACh is the neurotransmitter at the neuromuscular junction between the motor nerve and skeletal muscle. In the central nervous system, ACh is found primarily in interneurons, and a few important long-axon cholinergic pathways have also been identified. Noteworthy is the cholinergic projection from the *nucleus basalis* of Meynert (in the basal forebrain) to the forebrain neocortex and associated limbic structures. Degeneration of this pathway is one of the pathologies associated with Alzheimer's disease (AD)

AChE inhibitors or anti-cholinesterase inhibit the cholinesterase enzyme from breaking down ACh, increasing both the level and duration of the neurotransmitter action. According to the mode of action, AChE inhibitors can be divided into two groups: irreversible and reversible. Reversible inhibitors, competitive or noncompetitive, mostly have therapeutic applications, while toxic effects are associated with irreversible AChE activity modulators.

Reversible AChE inhibitors play an important role in pharmacological manipulation of the enzyme activity. These inhibitors include compounds with different functional groups (carbamate, quaternary or tertiary ammonium group), and have been applied in the diagnostic and/or treatment of various diseases such as: myasthenia gravis, AD, post-operative ileus, bladder distention, glaucoma, as well as antidote to anticholinergic overdose.

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DOI: <https://doi.org/10.5281/zenodo.3265296>

AD is a progressive neurological disorder, the most common form of dementia, characterized by memory loss and other intellectual abilities serious enough to interfere with daily life [7]. The disease is associated with loss of cholinergic neurons in the brain and the decreased level of ACh [8]. The major therapeutic target in the AD treatment strategies is the inhibition of brain AChE [8, 9]. AChE inhibitors consist in inactivation of the enzyme activity resulting in accumulation of synaptic ACh and consequent enhanced stimulation of postsynaptic cholinergic receptors in the central and/or peripheral nervous systems. According to the mode of action, AChE inhibitors can be divided into two groups: irreversible and reversible. Reversible, competitive or noncompetitive, inhibitors (donepezil, rivastigmine, glutamine) are protagonists in the pharmacotherapy of AD symptoms [10]. Ethno pharmacological approaches as well as bioassay guided isolation have given a lead to find possible AChE and BuChE inhibitors from plants including those for memory disorders [11].

MATERIALS AND METHODS

Standard Drug: Neostigmine

Test drug: Methanolic extract of leaves of *Ocimum basilicum*

Physiological solutions: Ringer Solution

Other chemicals: Acetyl choline

Animal: Frog (*Ranatigrina*)

Instruments: Sherrington Rotating Drum, Sterling's heart lever

Preparation of extract:

The leaves of *Ocimum basilicum* species collected from houses at Hanamkonda, Warangal District, and Telangana, India. It was authenticated by B.Raju Kakatiya university Warangal district. One specimen was preserved in Department of Pharmacognosy of our institute for the reference. The leaves were washed thoroughly to remove adhered material and fine powder was made by using hand grinder. 1gm of powder was mixed with 100ml distilled water with the help of magnetic stirrer for half an hour. The material was filtered through Whatman filter paper no.40 and filtrate was collected. The prepared infusion was diluted with the help of distilled water in varying proportions.

Rectus abdominus muscle contraction method:

The frog was pithed and laid on its back on the frog dissecting board and the four limbs were pinned. The skin on the abdomen was removed and the rectus abdominus muscle was exposed. The muscle was prepared and a thread was tied to the bottom and top of the each muscle preparation before detaching the muscle from the body of the frog. The preparation was mounted in upright position in organ bath containing frog ringer solution under a tension of 1g. The tissue was relaxed for 45 min with the wash of tissue with fresh quantum of ringer four times. The contractions of the rectus abdominus muscle due to the increasing doses of acetylcholine were recorded using either simple side way or frontal writing lever. The methanolic extracts of the different plants was mixed with the acetylcholine and the contractions and relaxation were recorded till a maximum response was reached. The height of the response was measured in mm and a dose-response curve was constructed.

RESULTS AND DISCUSSION

For the discovery of new drugs, natural products remain a prolific source and these new drug leads even from Vedic period. Recent data proposed that 80% drug molecules are natural products (NPs) or natural compound inspired. Herbal medicines are still the backbone of about 75 – 80% of the world population, mostly in the developing countries for primary health care because of their better compatibility and cultural acceptability with the human body and lesser side effects [12].

In recent years, the herbal medicines have been extensively used in various diseases because of their safety profile. When the Anti cholinesterase property compared with that of standard Neostigmine, the leaf extract of *Ocimum basilicum*, shows more Anti cholinesterase property than that of standard Neostigmine. Present study, 2µg & 4 µg of *Ocimum basilicum* extract significantly potentiates the action of acetyl choline that indicates 2µg & 4 µg shows good Anti cholinesterase property. In above 2µg & 4 µg of *Ocimum basilicum* leaf extract increasing the acetylcholine action when compared with that of Neostigmine, that indicates in above study, *Ocimum basilicum* leaf extract shows good Anti cholinesterase property than Neostigmine.

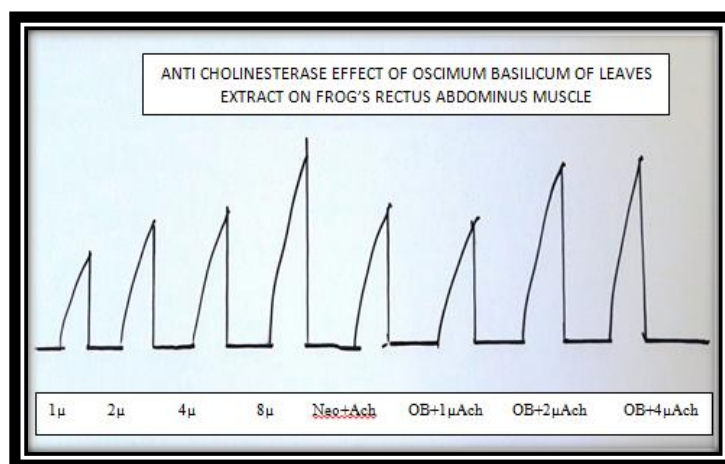


Fig. 1: Anti Cholinesterase effect of *Ocimum Basilicum* of Leaves extract on Frog's Rectus Abdominus muscle (Neo= Neostigmine; OB=*Ocimum basilicum*)

Table No. 1: Effect of *Oscimum Basilicum* on Frog's Rectus Abdominus muscle

Drug	Concentration (microgram)	Height (cm)
Ach	1	1.3
Ach	2	1.8
Ach	4	2.0
Ach	8	2.7
Std Neostigmine	2	2.0
Test+ach	1	1.7
Test+ach	2	2.5
Test+ach	4	2.6

CONCLUSION

The results of this study provide support for the traditional use of herbal leaf extracts shows Anti cholinesterase property, there is need to treatment for myasthenia gravis & reverse the effect of non depolarizing muscle relaxants, acute colonic pseudo obstruction & glaucoma. In market Anti cholinesterase drugs limitedly used, so there is a need to discover new drugs. However, further studies including toxicity studies are necessary to find the exact mechanism of Anti cholinesterase effect and to isolate the active compound responsible for this pharmacological activity.

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How to cite this article:

B. Rajendar, et al. EVALUTION OF ANTI CHOLINESTERASE ACTIVITY OF METHANOLIC LEAF EXTRACT OF *OCIMUM BASILICUM*. *J Pharm Res* 2019;8(6):383-386. DOI: <https://doi.org/10.5281/zenodo.3265296>

Conflict of interest: The authors have declared that no conflict of interest exists.

Source of support: Nils