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EFFICIENCY OF *BRAHMI* ON ACETYLCHOLINESTERASE ACTIVITY IN BRAIN OF ADULT WISTAR RATS

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ABSTRACT
Acetylcholine is a one of the prominent neurotransmitter of the peripheral and central nervous system which terminates synaptic transmission at cholinergic synapses by hydrolyzing the neurotransmitter acetylcholine and also exert morpho-regulatory effects on developing neurons such as stimulation of neurite outgrowth.The objective of present study was to evaluate the invivo effect of *Brahmi* on acetylcholinesterase (AChE) activity on adult wistar rat brain. *Brahmi* is a revered Ayurvedic medicinal plant used as nerve tonic since time immemorial. The result of present study suggests that *Brahmi* elicited higher acetylcholinesterase activity in rat brain.

KEYWORDS: Acetylcholinesterase, *Brahmi*, Acetylcholine.

INTRODUCTION
Acetylcholinesterase (AChE) convert acetylcholine to choline and acetyl-CoA and leading to the catalytic hydrolysis of neurotransmitter acetylcholine and the termination of the neurotransmission at cholinergic synapses both in central and peripheral nervous systems.(Laura et al., 2012, Rabindranath et al., 2013). Achetylcholinesterase is found in various types of conducting tissue which include nerve and muscle tissue, central and peripheral tissues, motor and sensory fibers, and cholinergic and noncholinergic fibers. (Wang et al., 2005). In vitro and in vivo studies have suggested that AChE enzyme in the central nervous system also plays a role in non-cholinergic functions like morphometric processes, cell differentiation and synaptogenesis along nervous system. (Silman et al., 2005).As mentioned above AChE plays important role in synaptic activity and any effect on it would compromise the transfer of information through synapse. Therefore effect on acetylcholinesterase activity is studied and to correlate it with cognition enhancing effect of Barhmi.

MATERIALS AND METHOD
Brahmi capsules manufactured by (The Himalaya Drug Company, Bangalore) was used for the study. The capsule was dissolved in water and used for the treatment. Stock solution of the drug was prepared in the sterile distilled water. The concentration of the drug was 150 mg per kg of body weight of the rat. Drug was administered orally. The control group of the rat was treated with the sterile distilled water in the similar manner. The drug was administered in the morning hours throughout the study period.

Animal procurement and management
Fresh stock of the male albino rats of wistar strain (weighing 130-180 grams and 5-8 weeks of age) were used for all the experimental work. All the animals were procured were obtained from Animal House, Bombay Veterinary College, BVC Campus Road, Parel, Mumbai 012, Maharashtra, India. All the animals were weighed and their health was verified. Animals were acclimatized to the experimental environment for a minimum period of eight days prior to the commencement of the study.

All experiments and protocols described in the present study were approved by the Institutional Animal Ethics Committee (IAEC) of RamnarainRuia College, Matunga, Mumbai 019, Maharashtra, India (CPCSEA/315).

Housing
All the animals were housed in polyurethane cages with wire mesh tops and rice husk bedding. The rice husk bedding was changed every day. Food and water was provided to the animal ad-libitum. Water was provided in an amber coloured glass bottle. A standard laboratory rat feed with balanced nutrition (crude protein 20-21%, crude fibre 4%, calcium1.2%, phosphorus 0.6%) was provided to the animals. The temperature of the animal house was maintained at 28°c (+/- 2°c). The animal house was provided with an artificial light at a sequence of 12 hrs light and 12 hrs dark cycles. Humidity of animal house was not controlled. The humidity as

recorded on humitherm was between 50-77% RH during the period of experiment.

Tissue preparation

Rats were sacrificed by decapitation. Rat whole brains were rapidly removed, weighted and thoroughly washed with ice-cold saline. Then washed with cold 0.1M phosphate buffered saline(PBS) pH7.4. Resuspended tissue in 500 – 1000 µL of ice cold 0.1M PBS. Homogenize tissue with a homogenizer or pestle sitting on ice, with 10 – 15 passes. Centrifuge sample 2 – 5 minutes at 4°C in a cold microcentrifuge to remove any insoluble material.Collect supernatant and transfer to a new tube.Supernatant was used as a source of enzyme.

The AChE activity was determined by following the hydrolysis of acetylthiocholine according to the method of Ellman, et al., 1961, Hestrin, 1949.The free thiol group of thiocholine reacts with 5,5'-dithiobis(2-nitrobenzoic acid) DTNB (Ellman's reagent) included in the assay mixture, producing the yellow 4 - nitrothiolate anion. The release of this yellow anion is measured at 412 nm.The statistical analysis was performed by using student's t-test.

RESULTS AND DISSCUSSION

The effect of Brahmi on acetylcholinesterase activity was studied on rats after 28 days of treatment. There was significant increase in the activity of acetylcholinesterase in treated rats as compared to the control rats given in Table 1. The treatment of animals with Brahmi for 28 days resulted in significant increase in the acetylcholinesterase(AChE) activity. In a study by Bhattacharya et al. (1999) they have stated that the Brahmi enhance the acetylcholine synthesis and this effect is attributed to its activation of acetylcholine synthesizing enzyme choline acetyltransferase, the increased level of acetylcholine may have caused the increase in the activity of acetylcholinesterase. Also in a study by Manish et al., 2013 have also reported that the enhanced synthesis and increase in the availability in the acetylcholine may be the reason for the increase in the acetylcholinesterase activity. Acetylcholine in the central nervous system is associated with attention, learning, memory, consciousness, sleep, and control of voluntary movements (Dimitri et al., 2008). Therefore the increase in the activity of acetylcholinesterase on treatment with Brahmi could be because of increase in the availability of acetylcholine which is in accordance to the previous studies and this could be one of the method by which Brahmi exhibit its memory enhancing effect. As mentioned above acetylcholine is associated with attention, learning, memory and earlier studies have shown that the primary mechanism of Brahmi is boosting the activity of acetylcholine synthesizing enzyme choline acetyltransferase therefore it is logical that Brahmi leads to the increase in the synthesis and availability of acetylcholine in response to which there is increase in the activity of acetylcholinesterase.

CONCLUSION

There was a significant increase in the activity of acetylcholinesterase on treatment with Brahmi, which may be indirectly helping in memory enhancing effect of Brahmi. Further studies are recommended.

DECLARATION

• Funding: None

• Competing interests: None declared.

Table no.1: Effect of Brahmi on Acetylcholinesteraseactivity.

Experimental Groups	Control	Treated
Mean	0.0016	0.0033
SE	± 0.000021	± 0.00048
Significance	$P \leq 0.01$	$P \leq 0.01$

The Acetyl cholinesterase activity in treated rats with the drug for 28 days were determined data represent mean ±SE, n=6 and $P \leq 0.01$.

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