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Research Article

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Nootropic activity of *Vigna aconitifolia* seeds in presence and absence of cognitive deficit mice model

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ABSTRACT

Dementia or cognitive problems are commonly seen in a large population. The factors such as emotions, stress and age are responsible for memory loss. In traditional system of Ayurvedic medicine, numerous herbs possessing saponins, have been used to treat cognitive disorder. Vigna aconitifolia (Fabaceae) is the rich source of saponins. Two doses (100 and 200 mg/kg., p.o.) of n-butanolic fraction Vigna aconitifolia extract (BVA) were administered for 7 successive days in separate group of animals. Furthermore, on the 7th day scopolamine (0.4 mg/kg., i.p.) administered to as cognitive deficit. The series of established neuropharmacological tests including elevated plus maze and passive avoidance paradigm were studied on 7th and 8th day. Baclofen induced hypothermia and measurement of acetylcholinestrase (AChE) activity were also carried out. BVA significantly improved learning and memory both in absence and presence of cognitive deficit. Also reversed the baclofen induced hypothermia. The mechanism by which BVA exerts nootropic activity was decreased AChE activity i.e. inhibition of AChE enzyme and inhibition of $GABA_B$ receptor. With the above data, we conclude that Vigna aconitifolia extract shows potent nootropic activity both in absence and presence of cognitive deficit.

Key words: Dementia, *Vigna aconitifolia*, saponins, Scopolamine.

INTRODUCTION

Memory is ability of an individual to record event, information and retains them over short or long periods of time and recalls the same whenever needed. Age, stress and emotion are conditions that may lead to memory loss or dementia [1]**.** Dementia is a mental disorder characterized by loss of intellectual ability sufficiently severe as to interfere with one's occupational or social activities. Dementia is of several types and it invariably involves impairment of memory. The most common cause of dementia is Alzheimer's disease, a progressive neurodegenerative disorder associated with loss of neurons in distinct brain area [2]. The central cholinergic pathways play a prominent role in learning and memory processes. Loss of cholinergic cells particularly in the basal forebrain, is accompanied by loss of the neurotransmitter acetylcholine. A decrease in acetylcholine in the brain of patients with AD appears to be a critical element in producing dementia [3]**.**

Recently, the mainstay treatments for the dementia are acetylcholinesterase inhibitors which increase the availability of acetylcholine at cholinergic synapses. AChE inhibitors from general chemical classes such as physostigmine, tacrine and galantamine like drugs have been tested for the symptomatic treatment of AD or dementia. However,

because of certain severe limitations of these drugs like non selectivity, their limited efficacy, poor bioavailability, adverse cholinergic side effects in the periphery, narrow therapeutic ranges and hepatotoxicity are among the severe limitations to their therapeutic success [4]**.** Therefore, in recent years, there has been a phenomenal rise in the interest of scientific community to explore the pharmacological actions or to confirm the veracity of claims made about herbs in the official book of Ayurveda.

Nootropics are the agents that enhance memory or cognition. They are drugs, supplements, nutraceuticals, and functional foods that are purported to improve mental functions such as cognition, memory, intelligence, motivation, attention, and concentration [5]. Several medicinal plants claimed to promote learning, memory and intelligence (nootropics). Plants like *Hypericum perforatum* [6], *Albizzia lebbeck* [7], *Asparagus recemosus* [8], *Tinospora cordifolia* [9], *Cissampelos pariera, Panax ginseng* as well as *Ocimum sanctum* [10] have been investigated for their effect on cognitive functions. Saponins from *B. monniera, P. ginseng* and *A. lebbeck* are active principles responsible for enhancing cognitive behavior in experimental animals. Since the seeds of *Vigna aconitifolia* (*Fabaceae)* are rich in saponins [11]. In the light of above information the present study was undertaken to investigate the effects of n-butanolic fraction of *Vigna aconitifolia* seeds (BVA) on cognitive functions activity in mice by using models like scopolamine induced amnesia, baclofen induced hypothermia and measurement of AChE activity.

EXPERIMENTAL SECTION

Collection of Plant materials

The seeds of *Vigna aconitifolia* were collected from the plants in local area near Aurangabad, Maharashtra, India. Sample was authenticated at the Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (voucher specimen no. 0538).

Preparation of plant extracts

Isolation of saponins

Saponins were isolated from dried seeds of *Vigna aconitifolia* as described by Pal et al [12]. Coarse powder of shade dried seeds of *Vigna aconitifolia* were defatted with petroleum ether (60–80^{0}C) in Soxhlet's extractor. The marc was dried and again extracted with Ethanol in Soxhlet's extractor. The Ethanolic extract was evaporated to dryness in vacuum. The residue was suspended in water, extracted with ethyl acetate and n-butanol and the solution was evaporated to dryness in vaccum to provide ethyl acetate n-butanol and water soluble portions. The n-butanol soluble fraction was tested for the presence of saponins using haemolysis test and foam test.

Experimental Animals

Albino mice (20-22 g) of either sex were used in this study. Those animals were allowed to acclimatize to laboratory conditions for 10 days after their arrival. The animals were housed in groups of six under standard housing conditions. Animals were fasted overnight prior to drug administration and during the experiment. All experiments were carried out during the light period (08:00-16:00 h). The experimental protocol was approved by Institutional Animal Ethics Committee (IAEC) of Y.B. Chavan College of Pharmacy Aurangabad (Approval number-CPCSEA/IAEC/P'col -14/2011-12/38.constituted as per the direction of CPCSEA, under Ministry of Animal Welfare Division, Government of India, New Delhi, India.

Drugs preparation and administration

Scopolamine (10 mg tablet/tab), baclofen (10 mg tablet/tab) and Piracetam (800 mg tablet/tab) is used as standard drug in this study. All drugs were dissolved in water. BVA extract was suspended in water by using span 80. Scopolamine, baclofen and piracetam were administered by intraperitoneal route and BVA was administered by oral route. All administered substances including BVA solutions and extract suspension were freshly prepared.

Experimental protocol:

All animals were randomly assigned to 8 groups (n = 6 in each group).
Group I: Vehicle treated (Normal control).
Group II: Piracetam treated positive control (200mg/kg).
Group III: Scopolamine treated (0.4mg/kg).
Group IV: BVA treated (100 mg/kg).
Group V: BVA treated (200mg/kg).

Group VI: BVA *(*100 mg/kg + Scopolamine) treated.
Group VII: BVA 200 mg/kg + Scopolamine) treated.
Group VIII: (Piracetam 200 mg/kg + Scopolamine) treated.

BVA Extract was administered by oral route for 7 days.

Behaviors evaluation:
The animals were divided into eight groups as mentioned earlier. The behavioral profiles were assessed for nootropic activity. Each animal was subjected to the following behavior task: (a) Elevated plus maze test (b) Passive avoidance paradigm.

Elevated plus maze
The Elevated plus maze (EPM) test is suggested to be a simple method for the evaluation of learning and memory in mice by measuring transfer latency. EPM served as exteroceptive behavioral model in which stimulus exist outside the body. An elevated plus maze consisting of two open arms (16cm x 5 cm) and two enclosed arms (16cm x 5cm x12 cm) were connected to give the apparatus a plus sign appearance was used. The arms extended from central platform (5cm x 5cm) and maze was elevated to the height of 25 cm. from the floor. On the first the day (7^{th} day of drug treatment), each mouse was placed at the end of open arm, facing away from central platform. Transfer latency (TL) was taken as the time taken by the mouse to move into any one of the covered arms with all its four legs. TL was recorded on the first day for the each animal. The mouse was allowed to explore the maze for another 2 min. and returned to its home cage. Retention of this learned task was examined 24 h after the first day trial (i.e. 8^{th} day of drug treatment).

Passive shock avoidance test
The nootropic activity was assessed using passive shock avoidance paradigm. Passive avoidance behavior based on negative reinforcement was used to examine the long-term memory. The apparatus consisted of a box (27 X 27 X 27 cm) having three walls of wood and one wall of Plexiglas, featuring a grid floor (3 mm stainless steel rods set 8 mm apart), with a wooden platform (10 X 7 X 1.7 cm) in the center of the grid floor. The box was illuminated with a 15 W bulb during the experimental period. Electric shock (20V AC) was delivered to the grid floor. On the first day(7^{th} day of drug treatment), The mouse was placed on the elevated platform, i.e. the shock free zone (SFZ), and the step down latency (SDL) was noted on the first day for each animal. Retention of this learned task was examined 24 h after the first day trial (i.e. 8^{th} day of drug treatment).

Baclofen-induced hypothermia
It is established that $GABA_B$ receptor function influences cognitive performance in the mammalian brain [13]. Baclofen-induced hypothermia model was used to assess the effect of drugs influencing GABA mediated behaviors [14].

Experimental protocol
Two groups of mice were used, **Group I:** vehicle + baclofen treated (Control)**, Group II:** BVA + Baclofen treated. Comprising of six animals in each groups were used in this study. BVA (100mg/kg) was administered and after 30 min baclofen (10mg/kg) was administered and rectal temperature was recorded using telethermometer after every 30 min interval till 180 min.

Estimation Acetylcholinesterase
On day ninth day, all mice were quickly decapitated by guillotine and brain was isolated from the skull immediately. The whole brain AChE inhibitory activity of extract was measured as described Ellman [15]. It is proposed that the prefrontal cortex, hippocampus and hypothalamus are important regions of brain involved in processing of memory and they are rich in cholinergic neurons. Briefly, the whole brain is homogenized in ice cold 0.1 M phosphate buffer (pH 8.0) using Remi cooling homogenizer. The homogenates were centrifuged at 10,000 rpm for 20 min at 4^0C, and supernatant was used as a source of enzyme in AChE assay. The total acetyl cholinesterase activity in the aliquot of the homogenate was estimated. The aliquot (0.3ml) was mixed with phosphate buffer (2.6ml) (pH 8.0). To this, the substrate acetylthiocholine iodide and dithiobisnitrobenzoic acid (DTNB) reagent were added. Acetylthiocholine iodide was hydrolyzed to thiocholine and acetate by AChE. Thiocholine react with DTNB reagent to produce a yellow color. The rate of formation of thiocholine from acetylcholine iodide in the presence of tissue cholinesterase was measured using a spectrophotometer. The rate of color development is the measure of the AChE activity.

Change in absorbance per minute of the sample was read at 412 nm. The enzyme activity is expressed as the 'n' moles of substrate hydrolyzed/min/mg of protein. The protein contents in the brain sample homogenates were determined using Lowry method [16].

Statistical analysis

All the results were expressed as mean ± standard error (SEM). Data were analyzed using one-way ANOVA followed by Dunnett's test and Tukey test. $p<0.001$ and $p<0.05$ were considered as statistically significant.

RESULTS

Elevated Plus Maze (In absence of cognitive deficit)

Transfer latency (TL) of the first day (on seventh day of the drug treatment) reflected acquisition or learning of information. Whereas TL of next day (2^{nd} day or 8^{th} day of drug treatment) reflected retention of information or memory. Mice treated orally with 100mg/kg and 200mg/kg of BVA showed remarkable reduction ($p<0.01$) in TL of 7^{th} and 8^{th} day, indicating significant improvement in learning and memory. Piracetam (used as standard) at the dose of 200mg/kg, i.p. also improved learning and memory in mice.

Elevated Plus Maze (In presence of cognitive deficit)

Transfer latency (TL) of the first day (on seventh day of the drug treatment) reflected acquisition or learning of information. Scopolamine hydrobromide (0.4mg/kg, i.p.) injected before training significantly increased ($p<0.001$) TL on days seven and eight indicating impairment in learning and memory. BVA at the higher dose (200mg/kg, p.o. for 7 successive days) successfully reversed memory deficit induced by scopolamine. Piracetam (standard) at the dose of 200mg/kg, i.p. also reversed the amnesia induced by SCOPOLAMINE.

Passive Avoidance Test (In absence of cognitive deficit)

Step-down latency (SDL) of second day (eighth day of the drug treatment) reflected the long term memory of animals. BVA at the doses of 100mg/kg and 200mg/kg administered orally for seven days markedly ($p<0.01$) increased SDL as compared to the control group. Piracetam (200mg/kg, i.p.) treated group of mice for seven successive days showed improvement ($p<0.01$) in memory of mice.

Passive Avoidance Test (In presence of cognitive deficit)

Step-down latency (SDL) of second day (eighth day of the drug treatment) reflected the long term memory of animals. Scopolamine (0.4mg/kg, i.p.) significantly decreased SDL as compared to control group, indicating impairment of memory (amnesia or cognitive deficit). BVA (100mg/kg and 200mg/kg, p.o.) administered for 7 successive days significantly reversed amnesia induced by scopolamine. Piracetam (200mg/kg, i.p.) treated group significantly reversed the amnesia induced by scopolamine.

Baclofen induced hypothermia in mice

Baclofen produced fall in rectal temperature from 35.58 ± 0.03^0 C to 32.87 ± 0.22 at 120 min. The peak hypothermic effect was observed 120 min after baclofen in the vehicle treated group. Pre treatment with BVA (100 mg/kg) significantly ($p<0.01$) inhibited the hypothermic activity of baclofen.

Effect of BVA on whole brain acetylcholinesterase level

Both the doses of BVA (100mg/kg and 200mg/kg) showed remarkable reduction in the whole brain AChE activity as compared to control group. Piracetam (Used as standard) at the dose of 200mg/kg, i.p., also reduced AChE activity in whole mice brain.

Effect of BVA on whole brain acetylcholinesterase level in presence of scopolamine

The whole brain AChE activity with Scopolamine (0.4mg/kg, i.p.) demonstrated significant rise in AChE activity as compared to control. BVA (100 and 200 mg/kg, p.o.) significantly ($p<0.001$) reversed the effect of scopolamine on whole mice brain and lowered the AChE activity. Piracetam (used as standard) at the dose of 200mg/kg, i.p. also counteract the effect of scopolamine on AChE, which facilitate the normal learning and memory processes.

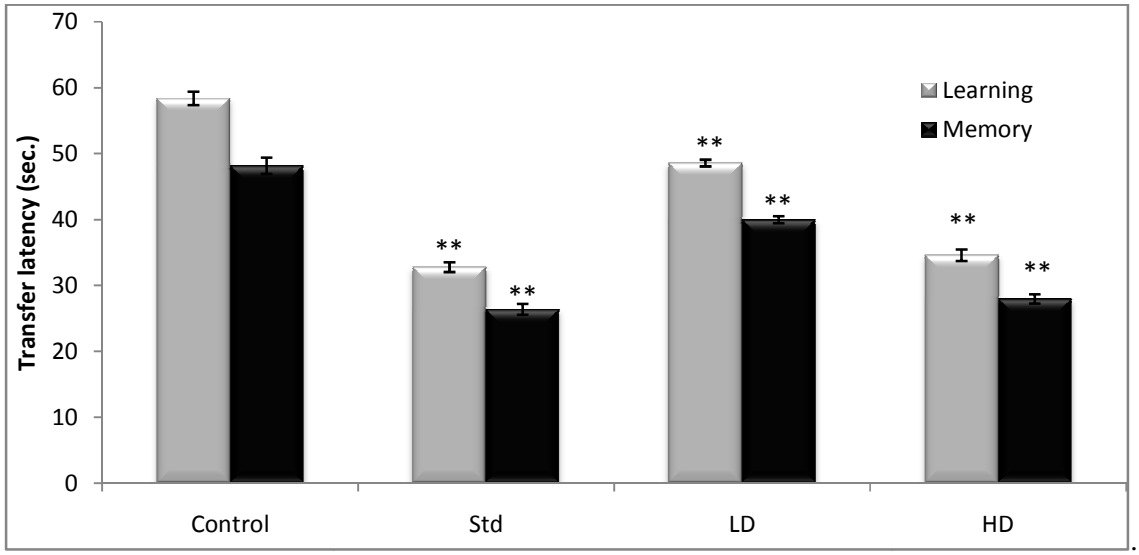

Fig. 1: Effect of n-butanolic fraction of *Vigna aconitifolia* (BVA) on transfer latency.
n = 5; values are presented as ± mean S.E.M.

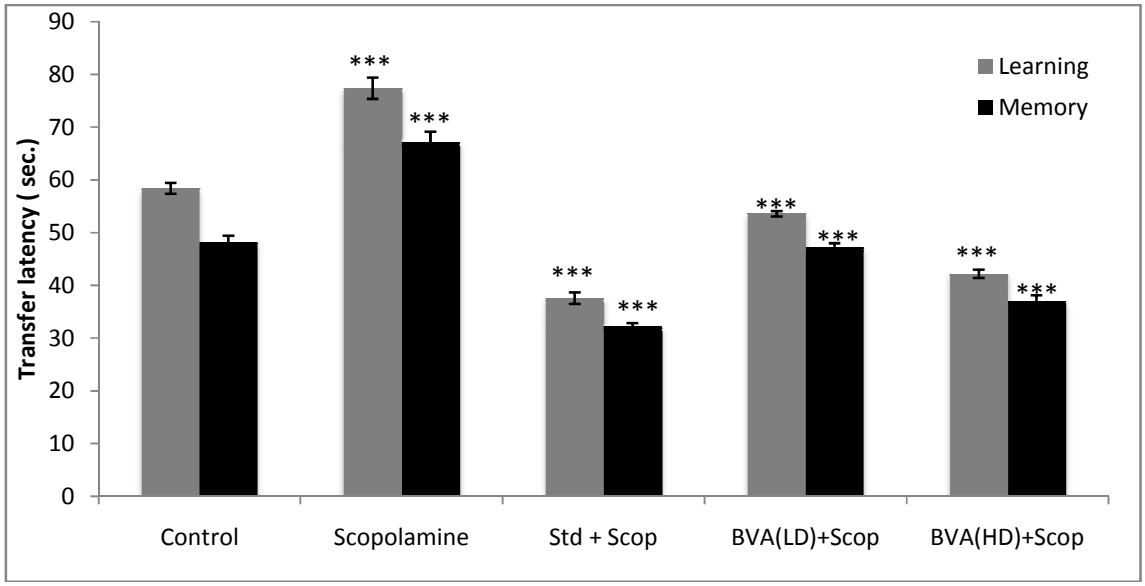

Fig. 2: Effect of n-butanolic fraction of *Vigna aconitifolia* (BVA) on transfer latency.
n = 5; values are presented as mean ± S.E.M.

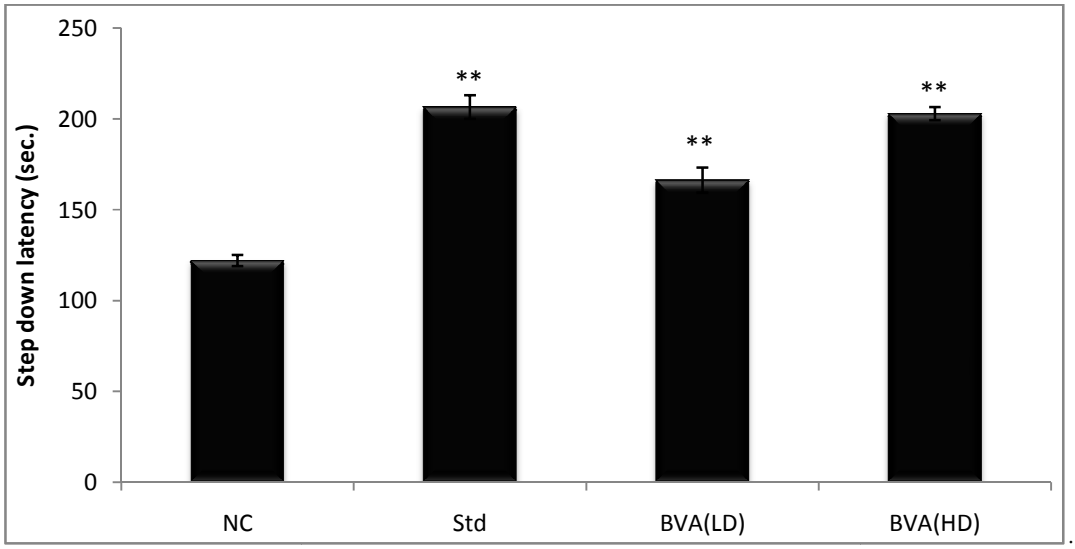

Fig. 3: Effect of of n-butanolic fraction of *Vigna aconitifolia* (BVA) on Step down latency.
n = 5; values are presented as mean ± S.E.M.

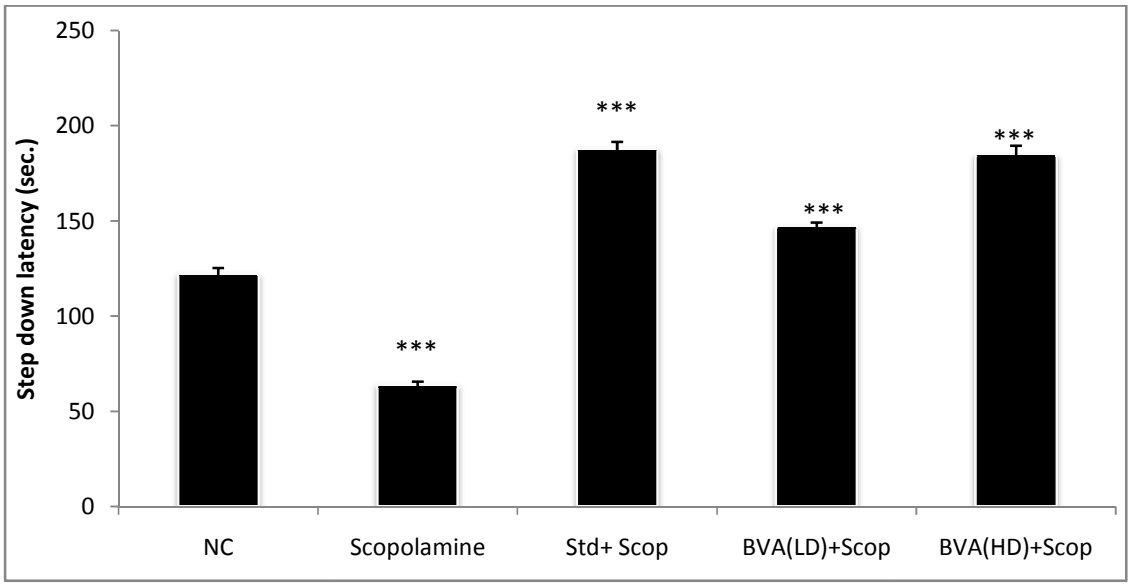

Fig. 4: Effect of n-butanolic fraction of *Vigna aconitifolia* (BVA) on Step down latency in presence of cognitive deficit. n = 5; values are presented as ± S.E.M.

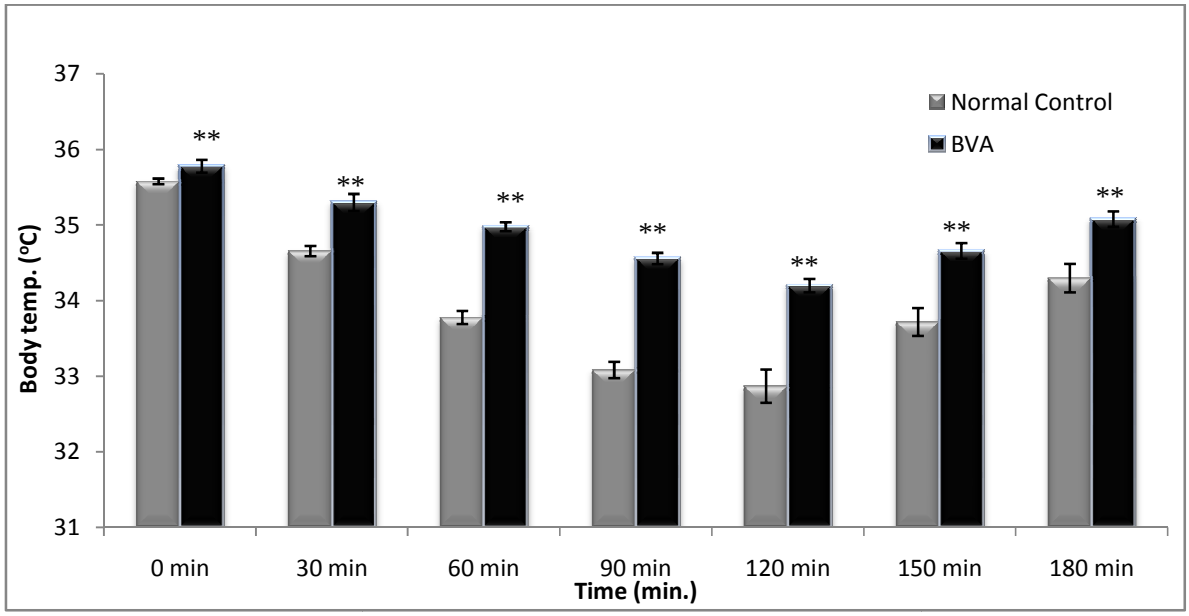

Fig. 5: Effect of n-butanolic fraction of *Vigna aconitifolia* (BVA) on rectal temperature.
n = 5; values are presented as mean ± S.E.M.

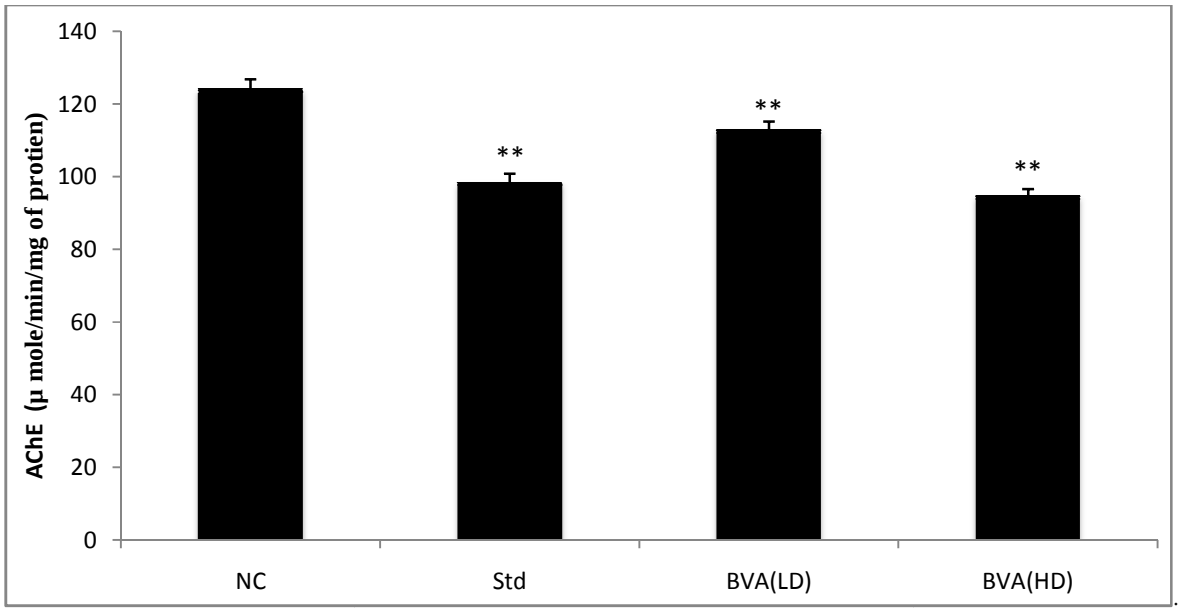

Fig. 6: Effect of n-butanolic fraction of *Vigna aconitifolia* (BVA) on brain AChE level.
n = 5; values are presented as mean ± S.E.M.

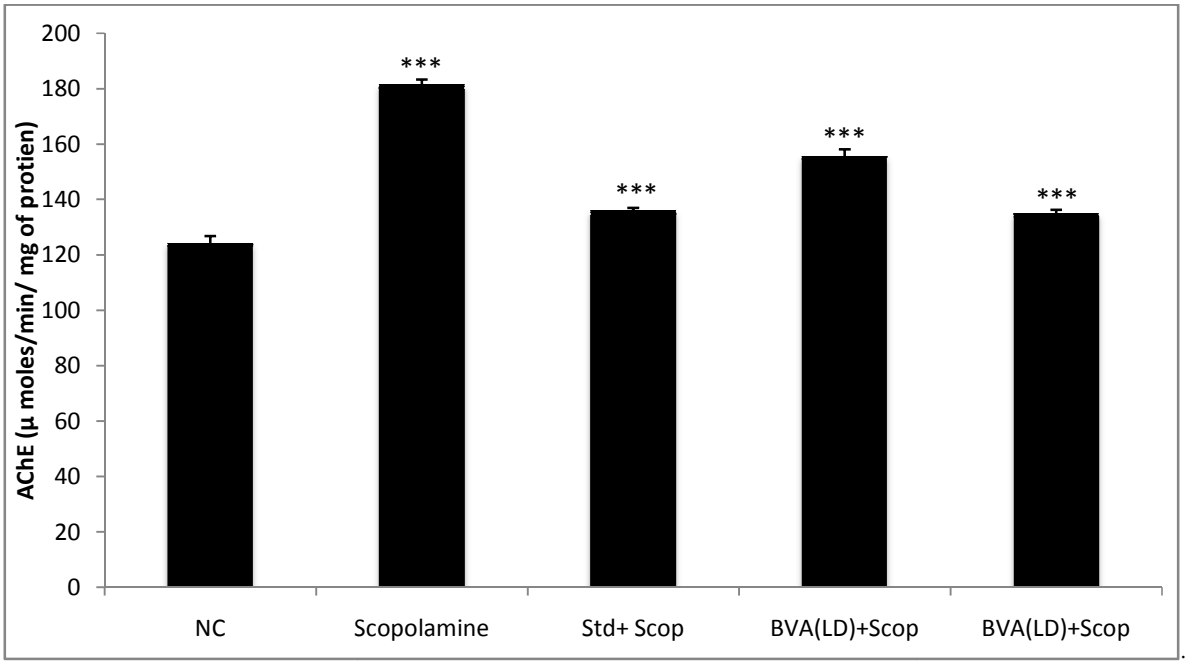

Fig. 7: Effect of n-butanolic fraction of *Vigna aconitifolia* (BVA) on brain AChE level.
n = 5; values are presented as mean ± S.E.M.

DISCUSSION

Dementia is a mental disorder characterized by loss of intellectual ability sufficiently severely interfering with occupational and social activity of an individual. It may be static, the result of a unique global brain injury, or progressive, resulting in long-term decline due to damage or disease in the body. Age, stress and emotion are conditions that may lead to cognitive dysfunctions, dementia and more ominous threat like Alzheimer's diseases. Alzheimer's disease is progressive neurodegenerative disease characterized by a progressive loss of memory and cognition. Despite the severity and prevalence of this disease, allopathic system of medicine is yet to provide a satisfactory drug [17]. Therefore, we were motivated to explore the potentials of medicinal plants to manage the dementia. *Vigna aconitifolia is* extensively used in Ayurvedic herbal medicine and in diets in India. It lacks scientific grounds for its neuropharmacological activities and to the best of our knowledge this is the first study to report its possible effects *in vivo* on the CNS.

Memory is the ability of an individual to record sensory stimuli, events, information etc., retain them over short or long periods of time and recall the same at a later date when needed [17]. The perception, learning, memory, and decision making, in other words, all ways in which animals take information about the world through the senses, process, retain, and decide to act on it can be called as cognition [18].

A number of drugs have now been introduced in therapy to ameliorate cognitive deficits. Nootropic drugs belong to the category of psychotropic agents with selective facilitator effect on intellectual performance, learning and memory [19]. The present study indicates that the n-butanolic fraction of seeds of *Vigna aconitifolia* (BVA) containing saponins, possessed nootropic activity in view of its facilitatory effect on retention of acquired learning in mice. The present study has attempted to correlate the $GABA_B$ antagonism with learning and memory. Nootropic activity assessed by using elevated plus maze (EPM) and passive avoidance paradigm, behavioral tests models (exteroceptive models).

EPM is a widely accepted model to study nootropic activity [20]. BVA administered orally for 7 days improved learning and memory of mice significantly, reflected by diminished TL (the time in which the animal moves from the open arms to the enclosed arms) and enhanced SDL values as compared to control animals. TL and SDL of 7^{th} day of treatment reflected acquisition or learning, whereas TL and SDL of 8^{th} day reflected retention of learned task

or memory. Thus, BVA extract meets a major criterion for nootropic activity, i.e. improvement of memory in absence of cognitive deficit [21].

After ascertaining the memory enhancing effect, we then tested using scopolamine-induced amnesic model (as interoceptive model) to find out whether BVA would be effective in reversing learning and memory deficits assessed by the plus maze and passive avoidance paradigm tests due to cholinergic perturbations. Scopolamine, is a non-selective muscarinic antagonist, induce a transient disruption of memory by blocking postsynaptic muscarinic receptors, [22] memory deficit produced by the scopolamine similar to those found in age related senile CNS dysfunction. Scopolamine interfere with memory and cognitive function and subsequently causes impairment of references (long term) and working (short term) memories [23]. This effect can be antagonized by cholinomimetics, such as physostigmine which increase brain Ach content. Cholinergic neurons in the central cholinergic system (CCS) possess an important function in the process of learning and memory [22]. Through augmentation of CCS function also associated with the pathogenesis of neurodegenerative disorders such as Alzheimer's disease (AD) [24].

Scopolamine, in the present study caused amnesia as observed by increased TL in elevated plus maze and decrease in SDL on passive avoidance paradigm. Pre-treatment for seven days with BVA at the doses of 100mg/kg and 200mg/kg dose-dependently protected the animals from memory deficits produced by scopolamine. These findings suggest the possible neuroprotective role for *Vigna aconitifolia.* Piracetam also revered scopolamine-induced amnesia in agreement with earlier reports [6].

Baclofen induced hypothermia was used to assess the effect of drugs influencing GABA mediated behaviors. It is well known that diazepam, a GABA mimetic drug induces memory impairment and the inhibition of $GABA_B$ receptor facilitates learning and memory. Baclofen, a $GABA_B$ agonist induces hypothermia, BVA inhibited the $GABA_B$ mediated behavior as indicated by diminished hypothermic effect of baclofen. Sarter have postulated that GABA antagonists may enhance cholinergic activity by blocking neurons that reach cholinergic nerve cells of basal forebrain [25].

To find out whether BVA has any central cholinergic activity, the effect of extracts was evaluated for Anti-AChE activity on whole brain of mice. Acetylcholine is considered as the most important neurotransmitter involved in the regulation of cognitive functions. According to the cholinergic hypothesis, memory impairment in patient with the senile dementia is due to selective and irreversible deficiency in the cholinergic functions in the brain [26]. This serves as rational for the use of AChE inhibitors for the symptomatic treatment of AD in its early stages. There are extensive evidences linking decreased cholinesterase activity and improvement in memory [6, 26, 27]. Cognitive dysfunction has been shown to be associated with impaired cholinergic functions and the facilitation of central cholinergic activity with improved memory [28]. Selective loss of cholinergic neurons and decrease in choline acetyltransferase activity was reported to be characteristic feature of senile dementia in AD [29]. Similar results were obtained in a study where *Taverniera cuneifolia* has enhanced memory and protected against scopolamine induced amnesia in mice models [30].

CONCLUSION

Our research findings have displayed a link between memory improving effect and cholinesterase activity. In the present study, effect of BVA on brain AChE was studied both in presence and in absence of cognitive deficit. Scopolamine (0.4mg/kg, p.o.) significantly elevated brain AChE activity. In the present study, BVA extract pre-treatment for 7 days (100 and 200 mg/kg, p.o.) inhibited AChE activity in Brain. These results suggest that BAV by virtue of its anticholinesterase property may significantly ($p<0.001$) enhanced cholinergic neurotransmission in brain and thus enhanced learning and memory functions. Pretreatment with BVA (at the dose of 100mg/kg and 200mg/kg) for 7 days significantly reversed the scopolamine induced rise in AChE level in mice whole brain which indicated neuroprotective effect of BVA.

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