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A STUDY ON NOOTROPIC ACTIVITY OF METHANOLIC EXTRACT OF *BRASSICA OLERACEAE* VAR. CAULORAPA BULB IN RODENTS

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Abstract

The present of the study was aimed to determine the nootropic activity of *Brassica oleraceae var. caulorapa bulb* methanolic extract rats.Methanolic extract of *Brassica oleraceae var. caulorapa bulb*(100, 200 and 400 mg/kg, oral.) and piracetam (200 mg/kg, *i.p.*) per se were administered to separate groups of rats. Effect of drugs on learning and memory of rats was evaluated using elevated plus maze and Morris water maze. Brain acetylcholinesterase concentration and its percentage of inhibition were also estimated.Methanolic extract of *Brassica oleraceae var. caulorapabulb*(100, 200 and 400 mg/kg, oral.) and piracetam (200 mg/kg, *i.p.*) on scopolamine induced and aluminuminduced amnesia models showssignificantly improved learning and memory of rats, as indicated by decrease in transfer latency using elevated plus maze and decrease in escape latency time during training and during retrieval using Morris water maze. Memory enhancing activity of methanolic extract of *Brassica oleraceae var. caulorapa* bulb (100, 200 and 400 mg/kg, oral.)was comparable to piracetam (200mg/kg, i.p.). Methanolic extract of *Brassica oleraceae var. caulorapabulb*(200 and 400 mg/kg, oral.) significantly reversed scopolamine and aluminum induced amnesia in rats. Methanolic extract of *Brassica oleraceae var. caulorapabulb*, Mentat (Nootropic Herbal formulation) were notably reduced the brain acetyl cholinesterase concentration and increased the percentage of inhibition of acetylcholinesterase activity in rat brain.This study conform the methanolic extract of *Brassica oleraceae var. caulorapabulb*memory-enhancing activity in rats probably by inhibiting brain acetylcholinesterase activity.

Keywords: Nootropic activity, *Brassica oleraceae var. caulorapa bulb*, scopolamine induced amnesia, aluminium induced amnesia and acetyl cholinesterase (AChE) estimation in brain.

Introduction

Dementia is devastating not only for those persons who have it, but also for their care givers and families. The total number of people with dementia worldwide in 2010 was estimated as 35.6 million and is projected to nearly double every 20 years, to 65.7 million in 2030 and 115.4 million in 2050. The total number of new cases of dementia each year worldwide is nearly 7.7 million, implying one new case every four seconds. It estimated that the numbers of people with dementia in India was 3.7 million in 2010 and this number is set to double in the next 20 years and will also experience rapid growth of 107%.The total estimated worldwide costs of dementia were US$ 604 billion in 2010 [1]. The commonest form of dementia is Alzheimer's disease (AD), mainly affects in older people. It is a neurodegenerative disorder characterized by cognitive and memory deterioration, progressive impairment of activities of daily living and a multiplicity of behavioral and psychological disturbances [2]. The primary causes of AD appear to be oxidative stress, deposition of amyloid-beta peptides in the brain anddecreased cholinergic activity. Acetylcholinesterase (AChE) plays a key role in the regulation of the cholinergic system and hence, inhibition of AChE has emerged as one of the major therapeutic strategies is to inhibit the AChE and hence, to increase the acetylcholine level in the brain [3]. The imbalance between the generation of free radicals and antioxidants has also been claimed to be a cause of AD [4].There are increasing evidences that increased consumption of fruits and vegetables and intake of certain non-nutrients that are present in foods reduce the risk of various pathological events such as cancer [5-6], neurological and cardio- and cerebro-vascular diseases [7]. The vegetables are rich sources of many nutrients and antioxidant vitamins such as polyphenols, vitamin C, vitamin E, carotene, and lycopene.The consumption of vegetables has been inverselyassociated with morbidity and mortality from degenerative diseases [8-12].*Brassica oleracea* species plants include many common foods as cultivars including cabbage, broccoli, cauliflower, kale, Brussels sprouts, collard greens, savoy, kohlrabi and kalian. Kohlrabi *(Brassica oleracea* variety *caulorapa)* "Kohlrabi" is a German word adopted without change into our language, *Kohl* meaning cabbage and *Rabi* meaning turnip. It contains polyphenols, tannins, flavonoids, iso thiocyanate glycosoides and alkaloids in the methanolic /crude extracts[13].Therefore, the objective of the study was to determine the nootropic activity of *Brassica oleraceae var. caulorapa* bulb methanolic extract using scopolamine induced amnesia, aluminum induced amnesia, morris water maze task & estimation of AChE models.

Materials and Methods

Chemicals

Piracetam (Intas pharamceuticals, India), Scopolamine butyl bromide (German remedies, Mumbai), Aluminum chloride (sarabhai chemicals Ltd), Acetylthiocholine iodide (ATCI), acetylcholinesterase enzyme (AChE) from bovine erythrocytes, 5,5`-dithiobis[2-nitrobenzoic acid] (DTNB) and galanthamine were obtained from Sigma Ltd (Mumbai, India) and methanol and all other chemicals and reagents used in the experiments were of analytical grade were used in this study.

Animals

Albino rats (170 - 210 g) of either sex were used in this study. Those animals were free access to food and water, and were maintained under standard laboratory conditions with alternating light and dark cycles of 12 h. The rats were fed with commercially available pellet diet and wereacclimatize to laboratory conditions for 10 days after their arrival. The animals were housed in groups of sixunder standard housing conditions. Rats 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 experimentalprotocol was approved by the Institutional Animals Ethics Committee (IAEC No.IAEC/SVCP/2012/001 and which is registered under the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), India.

Preparation of extracts

After selection of *Brassica oleracea* var. *caulorapa* (kohlrabi) bulb was washed under running tap water followed by washing with distilled water to remove the surface impurities. Exactly 500g of bulb was collected and weighed. The bulb was minced using a mixer grinder and finely macerated. After homogenization, macerates were extracted in 500 ml of methanol for 7 days at room temperature with intermittent shaking. After incubation, the whole extracts were filtered through filter paper and were maintained in the dark. 300 ml fresh methanol was then added and the mixture was refluxed for 90 min. The yield of crude extracts obtained from solvent was noted. The extractwas stored in desiccators for maximum of 3 days and later preserved in a deep

freezer (-20ºC) for experiment.

The preliminary phytochemical analysis

The preliminary phytochemical studies were performed for testing different chemical groups present in methanolic extract. Phytochemical screening gave positive test foriso thiocyanate glycosides, alkaloids. tannins, reducing sugars and flavanoids, and many other compounds.

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Toxicity studies

The parameters observed were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsion [19]. Acute toxicity studies was performed by using female Wistar rats (210 -250gms) 8 – 12 weeks old those maintained under standard husbandry conditions. The animals were fasted 3 hrs prior to the experiment, up and down procedure of CPCSEA was adopted for toxicity studies. The dose levels to be used as the starting dose is selected from one of the four fixed dose levels, 175, 550, 1750, 5000 mg/kg body weight. The treated animals were carefully observed individually after dosing at least once during the first 30 minutes, periodically during first 24 hours, with special attention for every four hours and daily there after, for a total of 14 days. The parametersobserved were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsion. There is nosigns of toxicity observed with the dose of 1750 mg/Kg body wieight.

Pharmacological screening

Scopolamine induced amnesia on elevates plus maze

Each consisting of six rats (n=6) weredivided into following groups

Group I: Control (vehicle used 2% gum acacia suspension)

Group II: scopolamine (0.5mg/Kg)

Group III: scopolamine (0.5mg/Kg) + Piracetam (200mg/Kg)

Group IV: scopolamine (0.5mg/Kg)+ *BOBME* (100mg/Kg)

Group V: scopolamine (0.5mg/Kg)+ *BOBME* (200mg/Kg)

Group VI: scopolamine (0.5mg/Kg)+ *BOBME* (400mg/Kg)

All the extracts (*Brassica oleracea* var. *caulorapa bulb* methanolic extract*)* were administered orally where as scopolamine and piracetam were given intra peritoneally, The rats were placed individually at the end of the open arm facing away from the center of the maze and at the time the rat took to move from open arm to either of the enclosed arms (transferlatency) was recorded. On before administration of extracts and drugs the rats were allowed to explore the plus maze for 20 sec for trial. On the day one after administration of scopolamine 30 minutes after extracts were administered and transfer latency was recorded. Extracts were administered continuously for 7 days. Transfer latency recorded on 1st day and 7th day. Retention or retrieval memory without extracts and drugs were recorded 24 hours later (8th day). The Mean ±SEM was calculated and the values pasted on tables and graphically represented.Results analyzed by one way ANOVAs followed by Dunnett' test.

Aluminium induced amnesia

Rats were randomly distributed in to 6 groups each consisting of six animals.

Group I:Control (2% gum acacia suspension)

Group II: Aluminium chloride(40mg/kg)

Group III: Aluminium chloride(40mg/kg) + Piracetam 200mg/kg (standard)

Group IV: Aluminium chloride (40mg/kg) + *BOBME* (100mg/Kg)

Group V: Aluminium chloride (40mg/kg) + *BOBME* (200mg/Kg)

Group VI: Aluminium chloride (40mg/kg) + *BOBME* (400mg/Kg)

Rats were administered aluminum chloride dissolved in distilled water (40mg/kg) administered once daily orally for period of 40 days. From day 21 of aluminum treatment, the drugs were administered once daily to different groups. Plant extracts administered orally to all the groups.

Rats were subjected to elevate plus maze on 40th day and 24 hours later on 41st day.The Mean±SEM was calculated and values are placed on tables and graphically represented too as on acquisition and retrieval memory. Results were analyzed using one way ANOVA followed by Dunnett's test.

Spatial memory in Morris water maze task

Rats were randomly distributed in 6 groups as follows.

Group I: control (vehicle used 2% gum acacia suspension)

Group II: scopolamine (SCP) (0.5mg/Kg)

Group III: scopolamine (0.5mg/Kg) + Piracetam (200mg/Kg)

Group IV: scopolamine (0.5mg/Kg)+ *BOBME* (100mg/Kg)

Group V: scopolamine (0.5mg/Kg)+ *BOBME* (200mg/Kg)

Group VI: scopolamine (0.5mg/Kg)+ *BOBME*

(400mg/Kg)

Apparatus

The Morris water maze consists of large circular tank made of black opaque PVC or hard board coated with fiber glass and resin and then surface painted white (1.8-2.0m in diameter and 0.4-0.6m height). The pool is filled with water (20-22^{0}C) to a depth of 0.3-0.4m and rendered opaque by the addition of small quantity of milk or non-toxic white colour. The pool is fixed with filling and draining facilities and mounted on a frame so that the water is at waist level. The floor of circular tank is marked off in to four equal quadrants arbitrarily designed north, south, east or west. And platform is made of plexiglass with a 13 cmsquare platform attached to a 34 cm long clearplexiglass cylindrical pedestal (3cm. Diameter) mounted on a 1sq.m (5mm thick) plexiglass base. Thetop of the platform is covered with a coarse materialthat provides a good grip for the rat when climbing ona platform. For the hidden platform task, water isadded to circular tank to a level 2cm above the top ofthe platform. Water maze represents a versatile tool inwhich a number of distinct tasks can be measured. Thesimplest measure of performance is the latency toescape from the water on to the hidden platform.

The platform remains fixed in the position during the training session. Each animal is subjected to four consecutive trials for four days during which they are allowed to escape on to the hidden platform and allowed to remain there for 20 sec. Escape latency time to locate the hidden platform in water maze is noted as an index of acquisition or learning. In case the animal is unable to locate the hidden platform within 20 sec, it is gently guided by hand to the platform and allowed to remain there for 20 sec.After trial session scopolamine and extracts were administered with 30 minutes of difference and subjected to task. The task was continued for every day up to 7 days. On 8th day without administration of drugs and extracts, platform is removed and time spent by each animal in target quadrant searching for the hidden platform is noted as an index of retrieval and measured [14].

Estimation of brain Acetyl Cholinesterase (AChE)

Rats were randomly distributed in 6 groups and each group of 6 rats for the estimation of Acetyl cholinesterase (AChE) andpercentage of inhibition of AChE.

Group I: Control (vehicle used 2% gum acasia suspension)

Group II: Piracetam (200mg/Kg) - Standard

Group III: Mentat (Herbal formulation) (200mg/Kg) - Standard

Group IV: *BOBME* (100mg/Kg)

Group V: *BOBME* (200mg/Kg)

Group VI: *BOBME* (400mg/Kg)

Acetylcholine is considered to be the most important transmitter involved in the regulation of cognitive functions, like learning and memory. Acetylcholinesterase inhibitors which enhance the availability of acetylcholine in the synaptic cleft. There are extensive evidences are present in the decrease of acetyl cholinesterase enhancement of memory. In this study we used a photometric method to determine the acetyl cholinesterase quantity in the brain tissue.The enzyme activity is measured by following the increase of yellow colour produced from thiocholine when it reacts with dithiobisnitro benzoate ion.

The reaction is Acetyl thiocholine → thiocholine + acetate Thiocholine + dithiobisnitrobenzoate → yellow colour The initial reaction is performed in the presence of acetyl cholinesterase enzyme.

The drugs and extracts treatment is continued for selected groups for 7 days. On the eighth day animals were euthanized by cervical dislocation carefully to avoid any injury to tissues. The whole brain AChE activity was measured using the Ellman method[15]. Ellman's reagent 5, 5-dithiobis (2-nitrobenzoate) is commonly known as DTNB. The end point was the formation of the yellow color because of the reaction of thiocholine with dithiobisnitrobenzoateions. The rate of formation of thiocholine from acetylcholine iodide in the presence of tissue cholinesterase was measured using spectrophotometer. The resulting yellow color is due to reduction of DTNB by certain substances in the brain homogenate and due to non-enzymatic hydrolysis of substrate. After having calibrated the instrument, change in absorbance per min of sample was read at 420 nm [16].

$$\text{Rate} = \frac{\text{Change in the absorbance / min}}{C_o} \times (5.74 \times 10^{-4})$$

Where,

Rate = Moles substrate hydrolyzed per min per gram of tissue

C_0= Original concentration of brain tissue (mg/ml)

Procedure

In- vivo determination of Acetyl cholinesterate quantity performed by homogentation of rat brain. Before sacrificing the animals extracts treated about 7 days. The whole brain taken out on 8th day and homogenized in the tissued homogenizer. (Approximately 20mg of tissue per ml of phosphate

buffer at pH 7.2). A 0.4 ml of this homogenate was added to cuvette containing 2.6 ml of phosphate buffer. To this 100µl of Ellaman's reagent added and then substrate (Acetyl thio choline iodide) added and absorbance measured at 412 nm. Values are expressed as Mean ± SEM. Statistical differences between means were determined by performing one-way ANOVA followed by Dunnett's 't' test. Significance at*P< 0.05, **P <0.01, ***P < 0.001 and ns-not significant. All the statistical analysis was performed using demo version of Graph pad 6.

Results

Transfer latency in scopolamine induced amnesia on elevates plus maze

Results were represented in table. 1. & figure.1. In this study, cholinergic muscarinic antagonist, scopolamine significantly increased the transfer latency on the first and seventh day, when compared to control group animals but on the eighth day, scopolamine induced transfer latency was drastically decreased when compared to the 1st day. This clearly indicates the learning behavior of animals on the seventh day. However, the nootropic agent, Piracetam showed significant reversal of scopolamine-induced deficits. Methanolic extract of *Brassica oleraceae var. caulorapa* bulbsignificantly and dose dependently decreased the transfer latency (TL) at 100mg/kg (P< 0.01), 200 mg/kg (P< 0.01) and 400 mg/kg (P< 0.01) as compared to Scopolamine administered rats on the seventh day. But on the eighth day, extract has shown same degree of effect on transfer latency on elevated plus maze at 100mg/kg (P< 0.05), 200 mg/kg (P< 0.01) and 400 mg/kg (P< 0.001) as compared to scopolamine administered rats. On eighth day, effect of extract with two different doses was almost same effect of standard drug (Piracetam).

Aluminium-induced cognitive deficits in rats on elevated plus maze

The results were shown in table .2 & figure.2. Aluminum chloride produced significantly increased the transfer latency on the 40th day, but on the 41st day Alcl3 induced transfer latency was slightly increased. This indicates no learning behaviour of animals with Alcl3 induced cognitive deficits on the 41th day. However, the nootropic agent, Piracetam showed significant reversal of Alcl3induced deficits. Methanolic extract of *Brassica oleraceae var. caulorapa* bulbsignificantly and dose dependently decreased the transfer latency (TL) on the 40th day. Effect of extract dose of 100mg/kg shown non-significant, where as 200 mg/kg and 400mg/kg was shown significant (P< 0.05) as with the effect of standard drug as compared aluminium chloride administered rats. But on the 41st day, extract has shown more degree of effect on transfer latency on elevated plus maze when compared to the 40thday effect.

Morris water maze task

The Escape Latency Time (ELT) was measured to assess spatial memory and results are displayed in table.3 &figure. 3. During pre-treatment session the test was conducted on the 1st day and we found that there was no significant difference in the ELT in all groups. On 4th day methanolic extract of *Brassica oleraceae var. caulorapa* bulb(*BOBME)* treatment as found to improve the spatial memory in a dose dependent manner with a significant reduction in the ELT at 100mg/kg (P< 0.05), 200 mg/kg (P< 0.01) and 400 mg/kg (P< 0.01) as compared to SCP rats. On 8th day *BOBME* treatment significantly reduced the ELT at 100mg/kg (p < 0.01), 200 mg/kg (P< 0.01) and 400 mg/kg (P< 0.01).

Estimation of acetylcholinestrase activity in rat brain

Control group had shown 10.48±0.22 µg/ml concentration of acetylcholinesterase in brain. Whereas piracetam 200 mg/kg, Mentat 200 mg/kg, *BOBME* 100 mg/kg, *BOBME* 200 mg/kg and *BOBME* 400 mg/kg showed decreased the acetylcholinesterase concentration as 8.72±0.229 µg/ml, 3.7±0.55 µg/ml, 10.71±0.23 µg/ml, 7.3±0.21 µg/ml and 4.95±0.29 µg/ml respectively.

There is increased in Percentage of AChE inhibition with piracetam 200mg/kg, Mentat 200mg/kg, *BOBME* 100 mg/kg, *BOBME* 200 mg/kg and *BOBME* 400 mg/kg as 16.85%, 64.60%, 1.12%, 30.33% and 62.35% compared with control group was found to improve the spatial memory in a dose dependent manner with a significant reduction in the ELT at 100mg/kg (P< 0.05), 200 mg/kg (P< 0.01) and 400 mg/kg (P< 0.01) as compared to SCP rats. On 8th day *BOBME* treatment significantly reduced the ELT at 100mg/kg (p < 0.01), 200 mg/kg (P< 0.01) and 400 mg/kg (P< 0.01).

Discussion

In this study *Brassica oleraceae var. caulorapa* bulb methanolic extract (100, 200 and 400 mg/kg, orally.) administered for 7 successive days showed significant memory enhancing effect in rats. Elevated plus maze and Morris water maze were employed as

behavioral models for evaluation oflearning and memory. These models are widely employed for evaluating the effect of drugs on learning and memory[17]. In elevated plus maze, decrease in transfer latency on 7th and 8th day indicated retention of memory and cognitive effects. In Morris water maze, a decrease in escape latency during training indicated improvement of learning and memory respectively. Out of the three effective doses of *BOBME* (100, 200 and 400 mg/kg, orally.), higher doses (200 and 400 mg/kg) produced better memory enhancing effect in rats (P <0.01) as compared to the lower dose (P <0.05) in both the behavioral models employed, hence the higher doses (200 and400 mg/kg) was employed for elucidating the probable mechanisms of memory enhancing activity. Scopolamine induced amnesia was reported in animal model for screening anti-amnesic molecules. In addition, this study also carried out in Alcl3 induced cognitive deficits model in rats to reconfirm the evaluation of extract activity of learning and memory. Aluminum exposure exerted adverse effects on learning and memory which were manifested in increases in the number of acquisition and retention errors of rats. Different studies also describe that aluminium exposure is a risk factor for the development of Alzheimer's disease (AD) in humans[18].

The signature lesions in Alzheimer's disease (AD) are neuritic plaques and neurofibrillary tangles (NFTs) located in the cortical areas and medial temporal lobe structures of the brain[19]. There are multiple neuronal pathways are destroyed in Alzheimer's disease (AD). Damage occurs in any nerve cell population located in or traveling through plaque laden areas [20]. Wide spread cell destruction results in a variety of neurotransmitter deficits, with cholinergic abnormalities being the most prominent [20]. Loss of cholinergic activity correlates with AD severity. In late AD, the number of cholinergic neurons is reduced, and there is loss of nicotinic receptors in the hippocampus and cortex. Presynaptic icotinic receptors control the release of acetylcholine, as well as other neurotransmitters important for memory and mood, including glutamate, serotonin, and norepinephrine [21]. Several clinical trials with cholinesterase inhibitors have consistently reported modest benefit in managing neuropsychiatric symptoms, although these are generally not the primary outcomes studied in the trials[22]. Memantine shows significant behavioral benefits for at least 6 months, either alone or in combination with cholinesterase inhibitors[22].

Vitamin Ebased on pathophysiologic theories involving oxidative stress and the accumulation of free radicals in AD, significant interest has evolved regarding the use of antioxidants in the treatment of AD. Vitamin E is often recommended as adjunctive treatment for AD patients. This recommendation is based on data from the published clinical trial, which evaluated the time to critical end points (i.e., death, institutionalization, loss of ability to perform activities of daily living, or severe dementia) in patients treated with 1060 psychiatric disorders vitamin E, selegiline, the combination, or placebo [23]. Central cholinergic system plays a major role in regulation of cognitive functions[24].Drugs that reducecholinergic function such as muscarinic receptor antagonist scopolamine produce amnesia in laboratory animals. In the present study scopolamine and aluminiumchloride significantly impaired memory of rats. Memory impairment effect of aluminium has been reported in the literature [18].

The vegetables are rich sources of many nutrients and antioxidant vitamins such as polyphenols, vitamin C, vitamin E, carotene, and lycopene[8-12]. *Brassica oleraceae var. caulorapa*contains polyphenols, tannins, flavonoids and alkaloids in the extracts[13]. *BOBME* (100, 200 and 400mg/kg, *orally.*) administered for 8 successive days in adifferent groups of rats significantly reversed scopolamine inducedamnesia and aluminiumchloride induced amnesia. Reversal of scopolamine and aluminium induced amnesia by*BOBME* indicated the possible facilitation of cholinergic transmission in central nerves system. *BOBME* (100, 200 and 400 mg/kg) also significantly reduced brain AChE activity in rats as compared to the control group. This suggests that the memory enhancing effect of *BOBME* might be due to inhibition of AChE, leading to increase in brain levels of acetylcholine andit also supported by the presence of vitamin E (antioxidents) in*BOBME* which will also help in nootropic activity. Acetylcholine is considered to be one of the important neurotransmitter involved in the regulation of cognitive functions. Cognitive dysfunction has been shown to be associated with impaired cholinergic transmission and the facilitation of central cholinergic transmission resulting in improved memory. Moreover, selective loss of cholinergic neurons in certain brain parts appeared to be a characteristic feature of senile dementia [25].

Thus, the drugs which enhance cholinergic function can be used for treatment of dementia closely related to AD. Oxidative stress has been shown to affect amyloid-beta generation in the AD pathogenesis. Thus, *BOBME* produced significant memory enhancing effect in rats probably due to its anticholenestrase and antioxidant property by increasing acetylcholine concentration in brain and by improving neuronal functions, later studies have shown that cholinesterase inhibitors also interact with cholinergic receptors, with sodium and potassium ion channels and effect the uptake, synthesis and release of neurotransmitters.

In conclusion, *BOBME* showed memory enhancing activity in rats probably by inhibiting brain acetylcholinesterase enzyme activity and increase neurotransmitter acetylcholine concentration via muscarinic cholinergic (Ach) receptors, which are implicated in memory process in brain and also through involvement its antioxidant activity.

Conflict of interest

The authors do not have any conflict of interests with the content of the paper.

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Table 1. Effect of *Brassica oleraceae var. Caulorapa* bulb methanolic extraction transfer latency in scopolamine (SCP) induced amnesia on elevates plus maze

S.No.	Groups	Transfer latency in sec. (Mean ± SEM)		
		1st Day	7th day	8th day
1	Control	37.66±1.2	36.33±1.3	30.66±0.42
2	Scopolamine treated (0.5mg/Kg)	88.33±1.3	108.0±2.8	61.33±1.9
3	SCP (0.5mg/Kg) + StdPiracetam 200Mg/kg	72.83±0.7 ns	29.16±1.16**	20.33±0.66**
4	SCP (0.5mg/Kg) + BOBME 100mg/Kg	83.66±1.2ns	29.5±0.89**	23.66±1.2*
5	SCP(0.5mg/Kg) + BOBME 200mg/Kg	77.83±1.16 ns	19.33±0.66**	14.33±0.6**
6	SCP (0.5mg/Kg) + BOBME 400mg/Kg	72.5±1.05 ns	11.5±0.42**	8±0.9***

n=6 in each group. Data expressed in Mean ±SEM, statistical analysis by one-wayANOVA followed by Dunnett's test. Significance at*P< 0.05, **P <0.01, ***P < 0.001 and ns-not significantVs SCP group and control group.

Table 2. Effect of *Brassica oleraceae var. caulorapa* bulb methanolic extract on Alcl3 induced amnesic ratsby elevated plus maze

Groups	Treatment(mg/Kg)	Transfer Latency (TL) in sec (Mean ± SEM)	
		40th day	41st day
I	Control	37.33±1.5	30.16±0.47
II	Alcl3 + Vehicle	51.5±1.02	59.5±1.28
III	Alcl3 + Std	28.33±4.7*	29.0±5.4*
IV	Alcl3 + BOBME(100mg/Kg)	35.83±1.74 ns	30±1.1 ns
V	Alcl3 + BOBME(200mg/Kg)	28.33±0.71 *	23.0±0.03 *
VI	Alcl3 + BOBME(400mg/Kg)	20.33±0.74*	15.16±0.60**

n=6 in each group. Data expressed in Mean ±SEM, statistical analysis by one-wayANOVA followed by Dunnett's test. Significance at*P< 0.05, **P <0.01, ***P < 0.001 and ns-not significantVs Alcl3 group control group.

Table 3. Effect of *Brassica oleraceae var.caulorapa bulb* methanolic extract on spatial memory in morris water maze task in rats

Group	Treatment	Escape latency (sec) (Mean ±SEM)		
		day 1	day 4	day 8
I	Control	47.33±0.88	31.5±1.05	15±0.96
II	Scopolamine (SCP)	51.66±1.08	47.36±0.60	46.16±0.87
III	SCP+Std(Piracetam) 200(mg/Kg)	43.83±1.16	18.83±0.60**	10.16±0.47**
IV	SCP+BOBME (100mg/Kg)	36.66±1.13 ns	25.66±1.25*	11.83±0.89**
V	SCP+BOBME (200mg/Kg)	35.66±0.49 ns	19.5±1.05**	9.33±1.28**
VI	SCP+BOBME (400mg/Kg)	34.08±0.79 ns	20.66±0.98**	4.83±0.70**

n=6 in each group. Data expressed in Mean ±SEM, statistical analysis by one-wayANOVA followed by Dunnett's test. Significance at*P< 0.05, **P <0.01, ***P < 0.001 and ns-not significantVs SCP group and control group.

Table 4. Estimation of acetylcholinesterase (in rat brain by Ellman's method 1960) in*Brassica oleraceae var.caulorapa bulb* methanolic extract administered rats

Groups	Treatment	Conc (µg/ml) Mean ± SEM	% percentage Inhibition of AchE
I	Control	10.48±0.22	-
II	Std(Piracetam) (200mg/Kg)	8.72±0.229	16.85%
III	Std(Mentat) (200mg/Kg)	3.7±0.55**	64.60%
IV	BOBME (100mg/Kg)	10.71±0.23 ns	1.12%
V	BOBME (200mg/Kg)	7.3±0.21**	30.33%
VI	B0BME (400mg/Kg)	4.95±0.29**	62.35%

n=6 in each group. Data expressed in Mean ±SEM, statistical analysis by one-wayANOVA followed by Dunnett's test. Significance at*P< 0.05, **P <0.01, ***P < 0.001 and ns-not significantVs control group.