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Neuroprotective Effect of *Brasicca Olarecae* L. Var. *Botrytis* (*Brassicaceae*) Flowers Extract on Memory Deficit in Aged & Young Rats

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Abstract

Ageing deteriorates memory, retention and recall in human beings and is associated with marked structural and neuro-chemical changes in the brain. The serotonin neurons in the hippocampus regulate memory. Low serotonin level is responsible for difficulty in making decision. Loss of the acetylcholine, neurotransmitter in the brain of patient with Alzheimer's diseases appears to be a critical element to produce senile dementia. Senile dementia is a clinical syndrome affecting the elder persons with loss of memory and cognition. Serotonin and acetylcholine, key neurotransmitters are made from respectively tryptophan and choline which are found in *Brassica oleracea* L. var. *botrytis* (cauliflower) vegetable also improve memory in people with Alzheimer's disease.

High levels of tryptophan in the brain directly influence increased serotonin production and new brain cell production begins to rise. The chloroform-metabolic extract of *Brassica oleracea* L. var. *botrytis* (CMEBOB) flowers was investigated for its neuroprotective effect against ageing related and scopolamine induced memory deficits in aged and young rats respectively using elevated plus maze and Morris water maze. 2000 and 3000mg/kg, p.o. doses of the extract were administered for four consecutive days. Oxidative stress biomarkers (elevated brain malondialdehyde, nitrite and lower reduced glutathione levels) in cognitive impairment of rats were significantly reversed by the flowers extract. Chronic consumption of CMEBOB extract (3000mg/kg, p.o.) significantly improved performance of both mazes in aged rats and scopolamine treated young rats by its potent cholinomimatic action, anti-oxidative action and could be useful in management of memory impairment associated with neurodegenerative diseases.

Keywords: Brassica; memory deficits; ageing; cauliflower; serotonin; acetylcholine; anti-cholinesterase; dementia

Abbreviations: AChE: Acetyl cholinesterase; Anti-ChE: Anti cholinesterase; BP: Boiling Point; CPCSEA: Committee for the Purpose of Control and Supervision of Experiment on Animal; DTNB:5, 5'-Dithio-bis 2- Nitro Benzoic Acid; ELT: Escape Latency Time; EPM: Elevated Plus Maze; GSH: Reduced Glutathione; CMEBOB: Chloroform-Methanolic Flowers Extract of *Brassica oleracea* L. var. *botrytis*; IR: Inflation Ratio; IVRI: Indian Veterinary Research Institute; MDA: Malondialdehyde; MWM: Morris Water Maze; NO: Nitric Oxide; Rpm: Round per minute; ROS: Reactive Oxygen Species;TBA: Thiobarbituric acid; TCA: Trichloro Acetic Acid; TLT: Transfer Latency Time; TSTQ: Time Spent in Target Quadrant

Introduction

Learning is defined as acquisition of information and skills. Subsequent retention of this information is called as memory. Any disturbance in dhi (process of learning), dhuti (process of retention) and smriti (process of recall) in Ayurveda results in the loss of cognitive ability [1]. Dementia, an organic disorder refers to several pathological states of brain, leading to disruption of personality and multiple higher cortical functions including memory, reasoning, orientation, comprehension, learning capacity and emotional stability [2]. It is therefore not a normal result of ageing, but rather pathological conditions [3] such as Alzheimer's disease, Pick's disease, cerebrovascular disease, hypoxic and ischemic encephalopathy, Parkinson's disease, alcoholism, drug abuse, brain tumor and infections like HIV and syphilis [4].

Vascular dementia is similar to senile dementia but memory is less affected, mood fluctuations more prominent; physical frailty, stepwise onset. Stroke-related dementia occurs when parts of the brain become damaged following a stroke. A stroke

occurs when blood supply to a part of the brain is suddenly cut off. This may cause difficulties in moving, problems with coordination, speech and sight depending on the part of the brain affected. If a stroke causes memory loss and problems with attention, then a person may be diagnosed with post-stroke dementia. Similar damage can also be caused by small strokes in the brain (which may be called transient ischemic attacks), multi-infarct dementia may be too small for a person to notice [5]. Marked fluctuation in cognitive ability; visual hallucinations; Parkinsonism are symptoms of Levy body dementia.

Personality changes; mood changes; disinhibition; language difficulties are symptoms of fronto-temporal dementia. The elder patient with Parkinson's disease characterized by rigidity, tremor, bradykinesia, and hypokinesia with secondary manifestations like expression less face, defective posture & shuffling gait due to degeneration of neurons in nigrostriatal dopaminergic tract and excessive secretion of saliva (sialorrhoea) and front temporal dementia due to increased cholinergic transmission, is more susceptible to the adverse effects (postural hypotension, ventricular dysrhythmias and psychiatric effects) of levodopa [6]. Prevalence rate of senile, vascular, lewy body and Fronto-temporal dementia is 50 to 70%, 20 to 30%, <5% and 5 to 10% respectively. The overall prevalence of late onset dementia is 7.1 per cent for people over 65. Today's memory impairment is major problem due to dementia in elder people.Ageing is a spiritual and psychological journey as well as a physical one. Ageing is commonly characterized by a progressive, generalized impairment of physiochemical and biological aspects of cellular body functions for an increasing vulnerability to environmental challenges and a growing risk of disease, long-term morbidity and potentially mortality [7]. As cells mature, they naturally stop dividing and enter into a period called cellular senescence. In response to DNA damage (shortened telomeres) cells either senescence or self-destruct (apoptosis), if the damage cannot be repaired. The concentration of oxidative damage proteins, lipids and DNA molecules increase in disease with the human ageing [8].

Ageing deteriorates memory, retention and recall in human beings and is associated with marked structural and neuro-chemical changes in the brain. The activity of choline acetyltransferase, a marker enzyme of acetylcholine, is reduced in neocortex due to accumulation of neurotoxin amyloidal beta protein and hippocampus due to formation of neurofibrillary tangles and consequently results in senile dementia. Senile dementia is a clinical syndrome affecting the elder persons with loss of memory and cognition [9,10]. Loss of the acetylcholine (ACh), neurotransmitter in the brain of patient with Alzheimer's disease appears to be a critical element to produce senile dementia.

ACh is synthesized in nerve terminals from acetyl coenzyme A and choline, and the reaction is catalyzed by choline acetyltransferase (ChAT). Scopolamine, a centrally muscarinic cholinergic receptor antagonist causes memory impairment along with reduced cerebral blood flow, increased acetylcholinestrase (AChE) activity and oxidative stress in rats and human's brain [11]. People diagnosed with amnesic MCI have an increased risk of developing dementia, but only about 1 in 6 eventually do. Functional memory problems: names of people, places; misplacing things; keeping track of schedule of commitments; forgetting to carry out an intended activity; numbers & passwords and remembering what was said or decided upon are commonly-known features of normal cognitive ageing, and also of mild cognitive impairment (MCI).

Its symptoms get worse with time. Amnestic' MCI is characterized by a set of problems with working memory, planning, language, and/or attention which decline more rapidly than they would in normal, age-related cognitive decline, but not to the extent that they interfere with a person's day-to-day life. Despite recent advances in our understanding of the pathophysiology of dementia, current treatments for these disorders often fall short of expectation. Patients, family members and caregivers often find themselves unable to resist the lure of using so-called natural products and dietary supplements as possible sources of new treatments to treat or prevent memory impairments and their behavioural and psychological symptoms [12].

Herbal medicines offer therapeutics for age-related disorder like memory loss [13]. Catechin and gallic acid from *Sanguisorbae radix*, epicatchin and 3, 4 dihydroxy benzoic acid from *Smilax rhizoma* are A β induced neurotoxicity inhibitors [14]. Therefore, it seems worthwhile to explore the utility of traditional medicines for the treatment of various cognitive disorders. The plants in *Brassicacaea*e family all share a common feature: their four-petaled flowers resemble to a Greek cross and are often referred to as crucifers or cruciferous vegetables.

This is a biennial and frost tolerant vegetable with compact heads of immature or aborted flowers contracted into a single white head. *Brassica oleracea* L. var. botrytis (cauliflower) family: *Brassicacaeae* has traditionally been found to boost memory in people with senile dementia. The name cauliflower comes from the Latin words *caulis*, meaning-stalk, and *floris*, meaning-flowers as suggested by its name; cauliflower is actually a flower [15]. Cauliflower is also an out-breeding plant. In addition, cauliflower must undergo vernalization in order to flowers. In some regions where winter temperature does not drop below 28F, brassicas can be planted, and seed is harvested the following summer.

Mostly, cauliflower is self- incompatible. Cauliflower rich in 27mg/100g tryptophan [16], indole-3-carbinol, vitamin B6, tocopherols, ascorbic acid, beta-carotene, kaempferol, quercetin, rutin, glycosides, fatty acid, cellulose, volatile oil, steroids, napthoquinone, vitamin-K, poly-phenols, cinnamic acid, sulforaphane and choline, the key ingredients make

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cauliflower such a powerful brain food. The allicin in cauliflower reduces the risk of strokes and improves the health of the heart. Selenium and vitamin-C work together to boost the immune system. The Indian home remedy of cauliflower is also used to treat inflammation, digestion, cardiovascular diseases, diabetes, neurodegenerative disorders, and various forms of cancers.

Choline intake during pregnancy "super-charged" the brain activity of animals in utero, indicating that it may boost cognitive function, and improve learning and memory. It may even diminish age-related memory decline and brain's vulnerability to toxins during childhood, as well as conferring protection later in life. The serotonin neurons in the hippocampus regulate memory and mood. Low serotonin level is responsible for difficulty in making decision. High levels of tryptophan in the brain directly influence increased serotonin production and new brain cell production begins to rise [17]. Tryptophan is the chemical precursor of serotonin and producing more serotonin through dietary means is more complicated than eating tryptophan-rich foods.

Vitamin B6 is needed to convert tryptophan to serotonin. ACh and serotonin, key neurotransmitters are made from respectively choline and tryptophan which are found in cauliflower vegetable also seem to improve memory in people with Alzheimer's disease. Therefore, the aim and objective of present research work is to investigate neuroprotective effect of *Brassica oleracea* L. var. *botrytis* flowers using interceptive (age related and scopolamine induced memory deficits) and exteroceptive (elevated plus maze and Morris water maze) behavioural models in young and aged rats and provide scientific basis for the same.

Materials and Methods

The experimental protocol was approved by the institutional animal ethics committee no.711/PO/Re/S/02/CPCSEA) and experiments were conducted according to the Committee for the purpose of control and supervision of experiment on animal (CPCSEA) guidelines on the use and care of experimental animals.

Plant material

The flowers of *Brassicaoleracea* L. var. *botrytis* was purchased from market. The flowers were authenticated by Dr. R. S. Saxena Reader and Head, Botany Department, Meerut College, Meerut. A voucher specimen was deposited for the plant in the same herbarium.

Extraction

Air dried flowers (20gm) of *Brassica oleracea* L. var. *botrytis* were coarsely powered and extracted with petroleum ether (B.P. 40-60 °C) for defatting of crude drug followed by chloroform: methanol (2:1) solvents by continuous hot percolation method using "Sox let apparatus"[18]. This was repeated thrice with fresh solvent each time. The extracts from all the three washes were pooled and concentrated using Rota-evaporator (Per fit) to obtain dark viscous mass. The residue was then dried at room temperature. The per cent yield of petroleum ether and the chloroform-methanol flowers extract was found to be 3.45 and 10 respectively. The extract was suspended in normal saline (0.9% w/v sodium chloride solution).

Drugs and chemicals

Piracetam and scopolamine hydro bromide drugs were purchased from Sigma Aldrich. Analytical reagent grade chemicals namely petroleum ether, methanol, acetyl thiocholine iodide, 5,5-dithiobis-2-nitrobenzoic acid (DTNB), trichloroacetic acid (TCA), triphenyltetrazolium chloride (TTC), thiobarbituric acid (TBA) were purchased from Fisher scientific.

Experimental Animals

Swiss albino rats of 30 months age (250+2g) and young rats of 6 weeks age (35+2g) were procured from Indian Veterinary Research Institute (IVRI), Izzatnagar, Bareilly (Uttar Pradesh) India. They were placed in animal house provided with 12 hours light and dark cycles at 25+2 °C and had free access to water and standard laboratory diet. Experiments were carried out between 09:00 and 17:00 hours. Efforts were made to minimize animal suffering and number of animals used.

Locomotors Activity Measurement by rota-rod

The rota-rod test is used to assess motor-coordination and balance in rodents. The ability of the rat to hold on to the horizontally rotating rod (diameter 2.5 cm, 4 rotations per minutes) was used to assess motor co-ordination [19]. Rat has to keep its balance on a rotating rod. It is measured the time (latency) taken by rat to fall off from the rotating rod. Latency at which each rat falls off the rod at different speeds from 4 to 40 rpm in 300sec was recorded. If a rat was climbing on the rod completed rotation and latency recorded. Rats which demonstrated impairment of muscle coordination with or without drug treatment were not included in the study. 15 % exclusion rate was noted.

Assessment of Neuro-protective effect

Interceptive behavioral models

Age-related memory deficits: An aged (30 months old) rat model for no tropic activity provides a natural model of ageing and cognitive decline and may be more translatable to humans-compared to other models which focus only on one particular aspect of the disease. Sensory perception becomes impaired with advancing age [20]. The aged rat expands the portfolio of options; drug developers have to study no tropic activity, getting us one step closer to discovering a cure.

Scopolamine-induced memory deficits

Scopolamine (0.3mg/kg, i.p.) is a central muscarinic receptor antagonist with memory deficits properties that have been used for decades in experimental young rats to induce impairment in

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their performance of a variety of tasks requiring intact working and reference memory [21].

Exteroceptive Behavioral Models

Elevated plus maze apparatus

Elevated plus-maze serves as the exteroceptive behavioral model to evaluate acquisition and retention of memory in rats [22]. The elevated plus maze for rats consists of two open arms (50 cm x 10cm) and two covered arms (50 cm x 10cm x40 cm) which are extended from a central platform (10cm x 10cm) and elevated to a height 50 cm from the floor. Acquisition of memory was recorded on day 4 after scopolamine administration. On the first day, each rat is placed at the end of an open arm, facing away from the central platform. Time taken by the rat to move into any one of the covered arms with all its four legs is recorded as TLT on the first day (i.e., day 4 of drug administration) for each animal. If the rats do not enter into one of the covered arms within 90 seconds, they are gently pushed into one of the two covered arms and the TLT is assigned as 90 seconds. The rat was allowed to explore the maze for 10 sec and then return to its home cage. Memory retention was calculated after 24 hrs of acquisition trial (on the day 5) as inflation ratio using the following formula:

Inflation ratio (IR) = L_1-L_0 / L_0

Where, L_0 is initial TLT on day 4 in seconds and L1 is the TLT after 24 hrs (day 5) of acquisition trial [23].

Morris water maze

Morris water maze was employed to evaluate learning and memory [24]. It consists of a circular water tank (diameter 150cm and height 45cm), filled with water maintained at 25 °C. The water is made opaque with a white coloured dye. The tank is divided into four equal quadrants with the help of two threads, fixed at right angle to each other on the rim of the pool. A platform (10 cm2) of 29cm height is located in the centre of one of these four quadrants. The position of platform and clues were kept consistent throughout the training session. In the present study, target quadrant was Q1.

Acquisition trials: Each rat was subjected to four consecutive trials on each day with an interval of five minutes, during which the rat was allowed to escape on the hidden platform and was allowed to remain there for 20sec. In case of the inability of rat to locate the hidden platform within 120seconds, it was gently guided by hand to the platform and allowed to remain there for 20sec. Escape latency time (ELT) to locate the hidden platform in water maze was noted as an index of acquisition and learning. In preliminary study, trials were conducted to familiarize the rat with the task and these trials were not counted. The rat was subjected to acquisition trials for four consecutive days. The starting position on each day to conduct four acquisition trials was changed as follows:

Day 1	Q1	Q2	Q3	Q4
Day 2	Q2	Q3	Q4	Q1
Day 3	Q3	Q4	Q1	Q2
Day 4	Q4	Q1	Q2	Q3

Retrieval trial: On the next day, platform was removed and each rat was allowed to explore the pool for 120 sec. Mean time spent by rat in each of four quadrants was noted. The mean time spent by the rat in target quadrant (Q4) for searching the hidden platform was noted as an index of retrieval. The experimenter always stood at the same position. Care was taken that relative location of water maze with respect to other objects in the laboratory, serving as prominent visual clues was not disturbed during the total duration of study.

Experimental Protocol

Table 1: Effect of CMEBOB on ELT during acquisition trials in scopolamine induced memory deficits. Control: I; Negative Control Group: II, III; Positive Control Group: VI to XV; Curative IV, V.

Group	Treatment	Dose (kg^{-1})	Day 1	Day 2	Day 3	Day 4
I	Control Group (0.9% NaCl)	10ml	108.63±6.65	91.88±16.24^{a}	63.17±15.25^{a}	40.63±15.08^{a}
II	Scopolamine in young rats	0.3mg	114.29±3.82^{b}	111.40±3.16^{b}	96.50±12.02^{b}	89.13±9.89^{b}
III	Ageing (0.9% NaCl)	10ml	120±00^{b}	105±1.41^{b}	90±0.70^{b}	85±0.52^{b}
IV	Piracetam- Per se treated young rats	200mg	101.84±10.50	62.10±7.91	36.84±8.81	16.50±4.37
V	Piracetam-Per se treated aged rats)	200 mg, i.p.	45.67±10.75^{b}	37.50±9^{b}	26.32±4.5^{b}	14±3.08^{b}
VI	CMEBOB - Per se in young rats	2000mg, p.o.	88.63±10.18^{b}	49.59±12.71^{b}	34±6^{b}	17.26±2.28^{b}
VII	CMEBOB Extract-Per se in aged rats	2000mg, p.o.	34.88±4.5^{b}	32.88±4.09^{b}	30.75±8.79^{b}	21.50±7.10^{b}
VIII	CMEBOB extract-Per se treated young rats	3000mg, p.o.	94.55±10.69^{b}	57.71±16.71^{b}	41.63±9.03^{b}	15.05±4.02^{b}
IX	CMEBOB-Per se extract treated aged rats	3000mg/Kg p.o.	33.57±6.03^{b}	28.69±3.69^{b}	28±2.03^{b}	26.82±7.79^{b}
X	Piracetam+ scopolamine treated Young rats	200+0.3mg p.o.	33±6.25^{c}	21.75±4.82^{c}	14.93±5.49^{c}	10±2.31^{c}
XI	Piracetam+ scopolamine treated aged rats	200+0.3mg p.o.	50±2.21^{c,d}	23±3.31^{c,d}	19.44±3.09^{c,d}	15.44±1.09^{c,d}

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XII	CMEBOB Extract (L) + Scopolamine treated Young rats	2000mg+0.3 mg, p.o.	67.37±20^{c}	61.69±9.19 ^{c}	55±8.35^{c}	27.44±8.85^{c}
XIII	CMEBOB Extract (L) + Scopolamine treated aged rats	2000m+0.3mg p.o.	79±1.79$^{c, d}$	68±3.75^{c,d}	40±3.44^{c,d}	25±11^{c,d}
XIV	CMEBOB Extract (H) + Scopolamine treated Young rats	3000mg +0.3 mg p.o.	48.06±14.92^{c}	38.26±5.75^{c}	30.57±5.92^{c}	18.19±3.99^{c}
XV	CMEBOB Extract (H) + Scopolamine treated Aged rats	3000mg+0.3 mg p.o.	73.50±14.24^{c,d}	50±2.11^{c,d}	31±3.55^{c,d}	28.15±4.59^{c,d}

Table 2: Effect of chloroform: methanolic extract of *Brassica oleracea* flowers on ageing and scopolamine induced decrease in time spent in target quadrant during retrieval trial.

Group	Treatment	Dose (kg^{-1})	Time Spent in Target Quadrant (Q1)
I	Control Group (0.9% NaCl)	10ml	50.41±4.89
II	Scopolamine in young rats	0.3mg	31±2.10^{a}
III	Ageing (0.9% NaCl)	10ml	31.37±3.48^{a}
IV	Piracetam-Per se treated young rats	200mg	74±5.16^{a}
V	Piracetam-Per se treated aged rats	200mg, i.p.	72±8.22^{c}
VI	CMEBOB-Per se in young rats	2000mg, p.o.	66±3.90^{a}
VII	CMEBOB Extract-Per se in aged rats	2000mg, p.o.	51.83±2.70^{c}
VIII	CMEBOB extract-Per se treated young rats	3000mg, p.o.	69±3.93^{a}
IX	CMEBOB-Per se extract treated aged rats	3000mg/Kg p.o.	63±4.81^{d}
X	Piracetam+ scopolamine treated Young rats	200+0.3mg, p.o.	49.5±4.68^{b}
XI	Piracetam+ scopolamine treated aged rats	200+0.3mg, p.o.	59±4.07^{b,c}
XII	CMEBOB Extract (L) + Scopolamine treated Young rats	2000mg+0.3mg, p.o.	61±6.51^{b}
XIII	CMEBOB Extract (L) + Scopolamine treated aged rats	2000mg+0.3mg, p.o.	47±2.57^{b,c}
XIV	CMEBOB Extract (H) + Scopolamine treated Young rats	3000mg+0.3mg, p.o.	69±4.80^{b}
XV	CMEBOB Extract(H) + Scopolamine treated Aged rats	3000mg+0.3mg, p.o.	55±3.56^{b,c}

Table 3: Effect of CMEBOB flowers extract on ageing related and scopolamine induced changes in Transfer latency time as Inflation ratio.

Group	Treatment	Dose (kg^{-1})	Inflation ratio
I	Control Group (0.9% Na Cl)	10 ml, i.p.	0.085±0.013
II	Scopolamine in young rats	0.3 mg, i.p.	0.021±0.008^{a}
III	Ageing (0.9% NaCl)	10 ml, p.o.	0.018±0.005^{a}
IV	Piracetam- Per se treated young rats	200 mg, p.o.	0.82±0.09^{a}
V	Piracetam-Per se treated aged rats	200 mg, i.p.	0.59±0.031^{c}
VI	CMEBOB - (L) Per se in young rats	2000mg, p.o.	0.318±0.21^{a}
VII	CMEBOB extract- (L) Per se in aged rats	2000mg, p.o.	0.225±0.016
VIII	CMEBOB extract - (H) Per se treated young rats	3000mg, p.o.	0.41±0.069^{c}
IX	CMEBOB- (H) Per se extract treated aged rats	3000mg/Kg p.o.	0.476±0.082^{c}
X	Piracetam+ scopolamine treated young rats	200+0.3mg, p.o.	0.298±0.048^{b}
XI	Piracetam+ scopolamine treated aged rats	200+0.3mg, p.o.	0.395±9.42^{b}
XII	CMEBOB Extract (L) + scopolamine treated young rats	2000mg+0.3mg, p.o.	0.225±0.016^{b}
XIII	CMEBOB Extract (L) + Scopolamine treated aged rats	2000mg+0.3mg, p.o.	0.318±0.034^{b,c}
XIV	CMEBOB Extract (H) + scopolamine treated young rats	3000mg+0.3mg, p.o.	0.228±0.014^{b}
XV	CMEBOB Extract (H) + scopolamine treated aged rats	3000mg+0.3mg, p.o.	0.352±0.179b,^{c}

The rats were divided into fifteen groups (Table 1-4). Each group comprised of six animals.In group I (control) & III, respectively normal rats and aged rats were administered 0.9 % w/v sodium chloride solution (10 ml/kg, i.p.) on day 1 and again after 24 hours i.e., on day 2. In group II and IV, young rats were administered scopolamine hydro bromide (0.3mg/kg, i.p.) and piracetam (200mg/kg) respectively. TLT was recorded after 45minutes in groups I, II & III; after 60min. in group IV respectively and then after 24hours i.e., on day 2) using EPM. In group VI and VIII of young rats, group VII and IX of aged rats were administered CMEBOB at doses of 2000 and 3000mg/kg, p.o. respectively for four consecutive days.

Table 4: Table showing the effect of *Brassica oleracea* L. var. *botrytis* on level of biochemical estimation.

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Groups	Treatments	Dose(Kg-1)	NO	AChE	Catalase	GSH	MDA	SOD
I	Control {Normal saline(0.9%Nacl)}	10ml, ip	0.0900	0.0000035	0.314000	52.3900	91.2900	638.6600
II	Ageing {Normal saline (0.9%Nacl)	10ml, ip	0.0140	0.0000053	0.325000	42.1700	98.9900	185.0000
III	Scopolamine	0.3mg, i.p	0.0008	0.0000079	0.390000	35.0600	148.2100	41.6000
IV	Piracetam	200mg, i.p.	0.6	3.600000 e-007	0.048	120.73	29.79	2740.25
V	Aging+Piracetam	200mg,i.p.	0.5550	4.900000 e-007	0.041600	114.7600	55.7300	3481.9300
VI	Extract	2000mg,p.o	0.3900	0.00000098	0.121000	85.2700	66.3600	1380.4000
VII	Extract	3000mg,p.o	0.5400	0.0000013	0.164000	88.0300	70.2000	1256.7800
VIII	Aging+Extract	2000mg,p.o	0.3800	0.00000079	0.071000	103.5900	64.4300	1421.6300
IX	Aging+Extract	3000mg,p.o	0.4200	0.00000098	0.085000	102.7600	65.4300	1339.1700
X	Piracetam +Scopolamine	200 + 0.3mg, i.p.	0.3700	2.500000 e-007	0.172000	69.2800	82.7600	885.9100
XI	Ageing+Piracetam +Scopolamine	200 + 0.3mg, i.p.	0.3800	0.0000015	0.184000	78.8200	97.1500	803.5100
XII	Extract +Scopolamine	2000mg, p.o + 0.3mg, i.p.	0.1500	0.0000017	0.271000	68.1300	74.0500	721.6000
XIII	Ageing + Extract (L)+Scopolamine	2000mg, p.o + 0.3mg, i.p.	0.1700	0.0000025	0.283000	65.3400	77.5700	721.0000
XV	Ageing+Extract(H) +Scopolamine	3000mg,p.o +0.3mg, i.p	0.3000	2.500000 e-007	0.150000	80.4100	73.3800	779.2500

In group VI to IX, TLT was recorded after 60 minutes on day 4 and then after 24 hours on day 5 using EPM. In groups X, XII, XIV of young rats and in groups XI, XIII and XV of aged rats were administered piracetam (200mg/kg, i.p.) and scopolamine hydro bromide (0.3mg/kg, i.p.); CMEBOB (2000 and 3000 mg/kg, p.o.) and scopolamine hydro bromide (0.3mg/kg, i.p.) respectively 60 and 45min. before the day 1 exposure on EPM. TLT was recorded on day 4 and then on day 5 in X to XV groups. Group I (control) & III, normal saline solution (10ml/kg, i.p.) treated respectively normal and aged rats was subjected to MWM for measuring ELT (from day 1 to day 4) and TSTQ on the day 5. In group II & IV, young rats were administered scopolamine hydro bromide (0.3mg/kg, i.p.) and piracetam (400mg/kg, i.p.) respectively before 45 minutes of acquisition trials conducted on four consecutive days (from day 1 to day 4). In case of VI and VIII, young rats, group VII and IX, aged rats, CMEBOB at doses of 2000 and 3000mg/kg, p.o., respectively were administered for four days before 60minutes of acquisition trials conducted on four consecutive days (from day 1 to day 4).

In groups X, XII, XIV, young rats, in groups XI, XIII and XV, aged rats were administered, CMEBOB (2000 and 3000mg/kg, p.o.) and scopolamine hydro bromide (0.3 mg/kg, i.p.); and piracetam (200mg/kg, i.p.) and scopolamine hydro bromide (0.3mg/kg, i.p.) 60 and 45min respectively, before the acquisition trials conducted on four consecutive days (from day 1 to day 4). In all the above mentioned groups, 0.9 % w/v sodium chloride solution (10ml/kg, i.p.) was administered 45 minutes before retrieval trial conducted on day 5.

Estimation of Oxidative Stress Markers in Brain Homogenate

On day 5 immediately after behavioural testing (retrieval), rat was euthanized by overdose of 2.5% thiopental sodium (100mg/kg, i. p.). It produced rapid induction with minimum excitation. The whole brain was carefully removed from the skull. For preparation of brain homogenate, the fresh whole brain was weighed and transferred to a glass homogenizer and homogenized in an ice bath after adding 10 volumes of phosphate buffer (pH 8, 0.1M). The homogenate was centrifuged using refrigerated centrifuge at 3000rpm for 10min at 4 °C. Drug induced lipid per-oxidation was measured by estimation of the content of MDA, nitrite, GSH, and AChE in brain blood sample of the rats. The determination was done by precipitating the protein substance using trichloroacetic acid (10% w/v), the protein free sample used for estimation of lipid per-oxidation parameters as follows:

Protocol for the estimation of brain malondialdehyde (MDA)

MDA was formed as an end product of lipid per-oxidation, which reacts with thiobarbituric acid and forms faint pink coloured trimethene complex. 1ml of supernatant was taken. 0.5ml of 30% of Trichloro acetic acid (TCA) and 0.3ml of 0.8%

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of thiobarbituric acid (TBA) were added in test tubes. The tubes were covered by the aluminum foil and then heated in water bath at 90 °C for 15min. Then the mixture was kept in ice cool water for 30min. After cooling, the absorbance of the colour supernatant in 1ml butanol was measured at 532nm using UV spectrophotometer [25]. MDA level was expressed in µM/mg.

Protocol for the estimation of brain Nitrite

The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide, was determined by a colorimetric assay with the Griess reagent (0.1% N-(1-napththyl) ethylenediamine dihydrochloride, 1% sulfanilamide and 5% phosphoric acid). Equal volumes of the supernatant and the Griess reagent were mixed and incubated for 10min at room temperature in the dark. The absorbance was taken at 542nm using a spectrophotometer. The concentration of nitrite in the supernatant was determined from a sodium nitrite standard curve [26].

Protocol for the estimation of brain reduced glutathione (GSH)

GSH was estimated by colorimetric method. GSH level was expressed in µM/mg of protein. The assay contained 1ml supernatant and 1ml of 10% of TCA. This mixture was centrifuged for 10min at 3000-4000 gyrations. Again the supernatant was collected and 10µl of supernatant, 2ml of 0.1 M phosphate buffer (pH-7.4), 0.5ml of DTNB (5, 5'-dithio-bis 2-nitrobenzoic acid) and 0.4 ml of distilled water were added and mixed. Absorbance of this mixture was measured at 412 nm within 15 minutes [27].

Protocol for the estimation of brain AChE activity

The resultant cloudy supernatant liquid was used for the estimation of brain AChE activity. The whole brain AChE activity was expressed in µM/min/mg of protein [28]. The end point was the formation of the yellow colour because of the reaction of thiocholine with dithiobisnitrobenzoate (DTNB) ions. The rate of formation of thiocholine from acetylcholine iodide in the presence of tissue cholinesterase was measured using spectrophotometer. The resulting yellow colour was 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, the change in absorbance per min of sample was read at 412nm. The rate of hydrolysis of substrate was calculated using following formula:

$$R = \text{change in absorbance/min} \times 5.74 \times 10^{-4}/C0$$

Where, R=rate of hydrolysis of acetylcholine iodide/min/mg tissue, C0=weight of tissue homogenate in mg/mL.

Statistical analysis

All the results were expressed as Mean±S.E.M. Data were analyzed by analysis of variance (ANOVA) followed by Turkey's test, Dennett's t test and student's and column static post-hoc tests using Graph Pad prism, version 5.03. The $p<0.05$ was considered as statistically significant.

Results and Discussion

Effect of CMEBOB on scopolamine and age induced changes in ELT during acquisition trials using Morris water maze model

Chloroform methanolic extract of *Brassica oleracea* L. var. *botrytis* (CMEBOB) flowers was investigated for its neuroprotective effect on scopolamine and age induced memory deficits using Morris water maze test. Scopolamine hydro bromide was administered in rats 30min before acquisition trials conducted on four consecutive days (day 1 to day 4) and ELT was noted as an index of acquisition and learning. Indeed, blockade of central muscarinic receptors could induce a pattern of cognitive impairment even in young rats. Scopolamine actions are limited to the blockade of brain function mediated via M_1-muscarinic cholinergic receptors.

Chloroform methanol extract of the plant drug was administered at 2000mg/kg, p. o. and 3000mg/kg, p. o. doses in rats. The ELT of CMEBOB flowers extract, conducted on four consecutive days are shown (Table 1). It is noted that the scopolamine and age have a significantly increasing effect on ELT. Chloroform methanolic extract of the plant drug and piracetam have a decreasing effect. Each value of escape latency time (ELT) is a mean value of four consecutive acquisition trials conducted from day1 to day 4 with a gap of 5minutes. Results were given in mean±S.E.M n=6 in each group. a=$p<0.05$ versus day 1 ELT in control group; b=$p<0.05$ versus ELT in control group on respective day, c=$p<0.05$ versus ELT in scopolamine treated group and d=$p<0.05$ versus ELT of aged rats. One way ANOVA followed by Turkey's multiple range tests.

A marked decrease in ELT of control group's rat during ongoing acquisition trials denotes normal acquisition of memory and an increase in time spent in target quadrant (Q1) in comparison with other (Q2, Q3 & Q4) quadrants to search missing platform during retrieval trial indicates retrieval of memory. No effect of normal saline solution employed in the present study to prepare solutions of drugs has been noted on acquisition and retrieval of memory. Therefore, the effect of pharmacological intervention on acquisition and retrieval trial of memory is due to plant drug and not because of its vehicle.

Effect of CMEBOB on scopolamine and age induced changes in time spent in target Quadrant (TSTQ) during retrieval trial using Morris water maze model

The effect of CMEBOB flowers extract at dose of 2000 & 3000mg/Kg was observed against scopolamine and age induced memory deficits in MWM. The TSTQ was assessed to evaluate the retrieval of memory during retrieval trial conducted on day 5. The plant drug showed protection against scopolamine and age induced memory deficits in rats (Table 2).

The effect of the administration of chloroform: methanol extract of *Brassica oleracea* flowers (CMEBOB) on time spent by rat in quadrant(s) during retrieval trial is exhibited. The

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time spent in quadrant (s) for control, vehicle (saline solution), standard drug (Piracetam), amnestic agent (scopolamine and age) and plant drug (CMEBOB) are shown. Note that while the amnestic agent and aged rats have a significantly decreasing effect on time spent in target quadrant (Q4), the standard and plant drug have a reversing effect. Each value represents mean time spent in quadrant (s) +S.E.M. a=p<0.05 Vs time spent in target quadrant (TSTQ) in control; b=p<0.05 Vs time spent in target quadrant (TSTQ) in scopolamine; c=p<0.05 Vs time spent in target quadrant (TSTQ) in aged rats.

Effect of CMEBOB per se on TLT as inflation ratio of young and aged rats

The effect of CMEBOB flowers extract per se on TLT at doses of 2000mg/kg, p.o. and 3000mg/kg, p.o. was observed against ageing related and scopolamine induced memory deficits in rats using elevated plus maze test. The dose dependent increase in the inflation ratio by CMEBOB flowers extract has proved that the plant possessed neuroprotective effect (Table 3). On the basis of results it can be concluded that CMEBOB flowers extract improves learning and memory. Thus, CMEBOB extract meets a major criterion for no tropic activity, namely, improvement of memory in absence of cognitive deficit. This observation has been strengthened by the finding that *Brassica oleracea* has increased the TLT as inflation ratio like piracetam indicating restoration of memory function [29].

Effect of CMEBOB on ageing related and scopolamine induced changes in TLT as inflation ratio

Chronic administration of cauliflower (2000 and 3000mg/kg/day for 4 days) significantly increased TLT as inflation ratio reversed ageing related and scopolamine-induced memory deficits in rats indicating improvement learning and memory (Table 3). The effect of chronic administration of chloroform-methanolic flowers extract of *Brassica oleracea* L. var. *botrytis* (CMEBOB) on transfer latency time (TLT) of rats is exhibited. The TLT for control, vehicle (saline solution), standard drug (Piracetam), memory deficits agent (scopolamine), aged rats and plant drug (CMEBOB) are shown.

Note that while the amnesic agent and aged rats have a significantly decreasing effect on TLT as inflation ratio, the standard and plant drug have a reverse effect. Results were expressed in means ± S.E.M with n=6 in each group. a=p<0.05 versus TLT of control group and b=p<0.05 versus TLT of scopolamine treated group and c=p<0.05 versus TLT of aged rats, one way ANOVA followed by Turkey's multiple range test.

The in-vivo study of *Brassica oleracea* L. var. *botrytis* was further supported by the estimation of biochemical parameter like brain AChE activity, GSH, MDA and NO estimations. Oxidative stress biomarkers (elevated brain AChE, MDA, nitrite, catalase and lower GSH and SOD levels) in age related and scopolamine induced memory deficit in rats were significantly reversed by the flowers extract (Table 4). Effect of *Brassica oleracea* L. var. *botrytis* on oxidative stress biomarkers in rat's brain homogenates was estimated spectrphotometrically.

As compared to control group in ageing and scopolamine treated group significant increase in AChE, MDA, NO and catalase levels but decrease in GSH, SOD levels were noted. Piracetam, *Brassica oleracea* L. var. botrytis (2000 and 3000mg/Kg, p. o.) treated aged group reduced the AChE, MDA, NO activity but increased GSH and SOD levels. AChE in M/min/mg, MDA, GSH, NO in M/mg, catalase in mg/min/mg and SOD level in Units/mg of protein were expressed. Coulomb static was followed.

Suloraphane, an isothiocynate sulfur containing component of the flowers is long lasting antioxidant and detoxifier, has been shown to stop over-rapid ageing by restoring antioxidant gene expression in human epithelial and promoting body's immune defense system. The evidence of cauliflower antioxidant properties may either prevent or delay chronic diseases associated with ageing [30]. CMEBOB flowers extract significantly reversed ageing related and scopolamine induced memory deficit in rats. Cauliflower is rich in tryptophan (precursor of serotonin) and choline, which may boost brain.

Choline exhibits cytoprotective and neuroprotective actions *in-vivo* and *in-vitro*. Cauliflower is reported to increase the formation and release of serotonin, choline and in brain and has allicin, potent antioxidant glycosides, fatty acid, cellulose, volatile oil, steroids, napthoquinone, vitamin-K, anti-inflammatory, immunostimulant and detoxification effects. Unique nutrition profile of cauliflower implies that it increases the resistance of the body against any onslaught. The underlying mechanism of neuro-protection may be attributed to its AChE inhibiting activity, anti-oxidant property, presence of tryptophan, cholineallic in and suloraphane.

Conclusion

Dementia accompanying ageing causes an obvious decline in the quality of life. Decreased levels of serotonin and choline in the brain have been reported to induce memory deficits and dementia due to increase in oxidative stress and AChE activity. Aged animals showed impaired learning and memory. Scopolamine (0.3mg/kg, i.p.) is a central anti-cholinergic drug that blocks muscarinic cholinergic receptors, consequently impairs acquisition and retrieval of memory along with reduced cerebral blood flow [31].

Chronic administration of chloroform: methanol (2:1) extract of *Brassica oleracea* L. var. *botrytis* flowers improved learning and memory, prevented age related and scopolamine induced experimental memory deficits in rats by inhibiting acetyl cholinesterase mediated hydrolysis of acetylcholine and oxidative stress as reflected by significantly increase in TLT as inflation ratio in EPM, decrease in ELT on four consecutive acquisition days and increase in TSTQ on day 5 in MWM respectively as compared to aged rats and scopolamine treated group.

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Drugs that inhibit AChE enzyme, protect acetylcholine from hydrolysis and may increase the level of acetylcholine neurotransmitters in hippocampus region of the brain and have no tropic activity [32,33]. The neuroprotective effect of *Brassica oleracea* L. var. *botrytis* flowers on memory deficits of aged and young rats may be attributed to its potential serotonin and cholinergic, anti-oxidative, anti-cholinesterase activity and could be useful as memory-restorative agent in treatment of clinical dementia of young and elder individuals.

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