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Memory enhancing activity of hydroalcoholic extract of *Brassica oleracea* on scopolamine induced amnesic rats

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ABSTRACT

Memory is the retention power of information in the brain. of *Brassica oleracea* commonly called 'Broccoli' has been known for its anti-inflammatory action. In this investigation, memory enhancing activity of *Brassica oleracea* is to be undertaken. Two doses of the drug *Brassica oleracea* extract (100 mg/kg and 200 mg/kg p.o) were selected after acute toxicity studies were subjected for the evaluation of cognition and memory enhancing paradigm against scopolamine (0.4mg/kg, i.p) induced amnesic albino rats. Piracetam (120 mg/kg i.p) served as standard drug for both the models (Elevated T maze, Passive avoidance test) undergoing investigation. Brain Anticholinesterase activity was observed as biochemical parameter for estimation. Both dose (100 mg/kg & 200mg/kg) of *Brassica oleracea* extract has shown dose dependent notable decrement in Transfer latency (TL) by EPM, reduced Anti Cholinesterase activity in brain signifies improved learning when compared to the test scopolamine group. Sub-acute long term treatment was found to be more significant than acute i.e. short term treatment on cognition and memory enhancing activity.

Keywords- *Brassica oleracea*, Cognition, Scopolamine Anti Cholinesterase, Amnesia

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INTRODUCTION

Alzheimer's disease, one of the most common disease of dementia, is a progressive, gradual neurodegenerative disease which is slow in onset which eventually, results in dementia, uncommon behavior, personality change and, finally leads to death. Creation of memory is the most complex process involving multiple neural pathways and neurotransmitters. It has been found out that the cholinergic nervous system plays a crucial role in learning and memory in humans and animals. [1]

Despite the great efforts and researches that have been made in understanding the disease and in management of these neurodegenerative diseases such as Alzheimer's disease (AD) but disease related complications are increasing unabatedly. A variety of synthetic drug therapies are available for the treatment of Alzheimer but they associated with severe side effects and are expensive. Therefore, it has become priority to explore all the available approaches to address the menace of this disease for controlling it. Alternative herbal approaches containing plant medication and herbal formulations are found of to be less toxic, with least side effects than synthetic one. Several plants such as *Azadirachta indica,*[2] *Bacopa monniera,*[3] *Withania somnifera*[4] as well as *Ocimum sanctum,*[5] are found to be very effective In spite of using synthetic medicines, herbal plants can be used to treat these disease with success.

Brassica oleracea commonly called 'Broccoli' belonging to genus Brassica is a vegetable known for its non-enzymatic bioactive compounds such as poly phenols, flavanoids, carotenoids, anthocyanin making it highly antioxidant in diet. This helps in reducing oxidative stress and is thus investigated in present study for its memory enhancing activity. [6]

Figure 1- Broccoli florets

MATERIAL AND METHODS

Animals: Sprang Dawley male albino rats weighing 150-180g were employed for study. They have *ad. libitum* access to water food pellets (Hindustan Lever Ltd., Mumbai) maintaining the standard controlled room temperature (24±2°C) with alternating 12-h light-dark cycle in groups of three to four per cage. The experiment was carried out in a quite environment between 8.00 and 12.00 am. Approval was granted from the Institutional Animal Ethics Committee, Modern Institute of Pharmaceutical Sciences, Indore, India for conducting the study.

Acute toxicity study: The acute toxicity was performed as specified in OECD 423 guidelines. [7] The extract suspension, at the dose of 5,50,100, 300 and 2000 mg/kg body weight, was dispensed orally to the rats and were subsequently observed closely for the first 4 h for any unusual behavioral symptoms such as salivation, tremors, exophthalmus, convulsions, lethargy & diarrhea followed by observation for a further 14 days, the animals were observed for any changes in behavioral pattern and mortality. One tenth of the safe dose was selected for the study.

Chemicals: Scopolamine, Acetylcholine(Sigma Laboratories, Mumbai, Maharashtra, India), Piracetam (UCB India Ltd., Mumbai, Maharashtra, India), simvastatin (Krebs Biochemicals and Industries Limited, Hyderabad, India.

Drugs administration: *Brassica oleracea* purchased from the local market of Indore and were authenticated at the Government Agriculture College, Indore, India. Floret were separated and were dried under shade and hydroalcoholic (70:30) extract was prepared under soxhlet assembly for 48 hours. Then, the extract was dispensed orally to the rats at two doses of 100 and 200 mg/kg/day daily at the same time suspended in 1% CMC solution.

Laboratory Models for Testing Learning and Memory

(i) Elevated plus-maze.

(ii) Passive shock avoidance paradigm.

(iii) Biochemical estimation

Experimental protocol: the rats were trained daily at same time for 15 days then the rats were divided into two sets containing eight groups each (16 groups) with treatment period of 7 day and 14 days respectively. Except for the vehicle control group, all the animals were given scopolamine on 7th and 14th day after 1 hour of drug administration. After its 45 minutes, cognitive parameters were analyzed using EPM, and passive avoidance. This trail conducted is called as Acquisition Trial (AT), which corresponds to learning. Again after 24 hr the same procedure is followed using models EPM, passive avoidance test which will be termed as Retention Trial (RT) which corresponds to retention of learning or memory. Further, for biochemical estimation the rats were euthanized by cervical decapitation and their brains were isolated to evaluate the anticholinesterase (ChE) activity of *Brassica oleracea.* [8]

Exteroceptive behavioral models

EPM: The EPM[9,10] used to evaluate anxiety & cognition ,is a elevated maze at height 50 cm above ground, it has two open (50 cm × 10 cm × 40 cm) ,two enclosed arms (50 cm × 10 cm × 40 cm),and an open roof, arranged in a manner that the open arms were opposite each other.

On the 7th and 14th days, respectively to analyze the learning and memory retained, each rat was placed at the end of an open arm, in the opposite direction to the central platform, the time taken by rat to move and enter in the other closed arm is termed as Transfer latency (TL) is recorded. If the rat did not enter in other closed arm in 180 seconds, they are pushed into one of the two closed arms and in this case TL was assigned to 180 s. The rats was allowed to move in the maze for 15 seconds and then are returned to their cage. After 24 hours to calculate RT or memory retained again TL is analyzed on 8th and 15th day.

Passive shock avoidance paradigm: Passive avoidance, [9, 10] I a fear aggravated test used to evaluate long term memory . The apparatus is box (27 cm x 27 cm x 27 cm) having three wooden walls and a plexiglass wall, with a grid floor (consisting of 3 mm stainless steel bars spaced 8 mm apart), with a platform (10 cm × 7 cm × 1.7 cm) in the middle of the grid floor. The box was lit with a 15 W bulb during the experimental period. The electric shock was applied to the floor of the box. The rats were initially trained in the following manner: each rat was placed on a wooden platform in the center of the floor of the grid. When the rat steps down and places the paw on the floor of the grid, a discharge was administered (foot discharge: 50 Hz, 1.5 mA, 1 s) and the Step Down Latency (SDL) was recorded. SDL is defined as the time taken by rat to step down to keep all 4 paws on the grid floor. Rats showing SDL in the range of 2 to 15 seconds during the training session were taken for acquisition and conservation activities. The acquisition operation was performed 90 minutes after the training session. During the acquisition test, the animals were removed from the area without discharge if they were not removed for a period of 60 s. Retention was tested after 24 hours in a similar manner, except for a cut-off time of more than 180 s.

Interoceptive behavioral models

Estimation of ACh Levels in the Brain by Quantifying ChE Inhibition:

After evaluating the learning and memory paradigms, the rats in each group were sacrificed by cervical decapitation. The entire brain was removed immediately and cooled in frozen phosphate buffer. After washing in cold phosphate buffer, the brains were homogenized in 5 ml of phosphate buffer in a TEFLON glass homogenizer. The enzymatic activity of the cerebral homogenate was then evaluated using the Augustinsson analysis method .[11]

RESULTS

Acute toxicity profile: Rats treated with *Brassica oleracea* extract, 5-2,000 mg / kg, p.o., showed normal behavior. They were alert, with a normal grooming, response to touch and response to pain. There were no signs of depression, stereotyping and vocalization. The alertness, the tone of the extremities and the strength of grip and the walking of the animals were normal. The *brassica oleracea* suspension was safe up to a dose of 2,000 mg / kg in rats.

Exteroceptive behavioral models

Elevated Plus Maze Model: On 7th and 14th day, at the end of treatment period, the effect of the vehicle, scopolamine control, *Brassica oleracea* (100 and 200 mg/kg) and Piracetam (120 mg/kg) were analyzed. The scopolamine control group (0.4 mg / kg) showed notable increase ($P < 0.01$) in TL of acquisition and on retention as compared to vehicle control rats, indicating a impairment in learning and in memory. At AT on day 7 for set I and day 14 for set II and At RT of day 8 for set I and day 15 for set II, *Brassica oleracea* at dose levels of 100 and 200 mg / kg showed a significant dose dependent decrease ($P < 0.01$) in TL compared to control group scopolamine. Piracetam (120 mg/kg p.o.)

exhibited significant decrease ($P < 0.01$) in TL in comparison with the scopolamine control group. However, *Brassica oleracea* at the dose levels 100 and 200 mg/kg showed a decrease in the TL, which is comparable to that shown by Piracetam ($P < 0.01$) **[Table 1]**

Table 1: Effect of the extract of *Brassica oleracea* on transfer latency (Elevated Plus Maze paradigm) in scopolamine-induced amnesia in rats

Treatment Groups	TL on acquisition day (sec)		TL on retention day(sec)	
	7 day	14 day	7 day	14 day
Vehicle control	94.17±14.96	42.83±5.80	54.33±8.75	23.93±4.11
Scopolamine hydrobromide	147.77±10.2	114.5±11.13	125.13±19.23	117.83±26.23
Brassica oleracea (100)+ Scopolamine (0.4)	61.17±7.48	32.18±7.75	44.26±8.17	12.24±2.12
Brassica oleracea (200)+ Scopolamine (0.4)	53.5±6.65	24.71±3.05	34.27±5.61	13.53±2.02
Piracetam(120)+ Scopolamine (0.4)	52.17±9.81	23.89±3.61	32.81±1.71	11.75±0.30

Values are expressed as mean ± SEM at n=6; one way ANOVA followed by Dunnett's Test

Passive shock avoidance paradigm: The scopolamine hydrobromide (0.4 mg / kg i.p.) reduced SDL in AT and RT training, indicating a deterioration of memory. There is a slight increase in SDL after administration of Brassica oleracea (100 and 200 mg / kg po) for 7 days compared to the scopolamine control group on AT and RT, indicating an improvement in learning and rat's memory. It was found that *Brassica oleracea* (100 and 200 mg / kg) significantly reduced (P <0.05) SDL in RT compared to scopolamine control after 14 days of administration **[Table 2]**

Table 2: Effect of the paste of *Brassica oleracea* on step-down latency (passive avoidance paradigm) in scopolamine-induced amnesia in rats

Treatment Groups	SDL on acquisition day (sec)		SDL on retention day(sec)	
	7 day	14 day	7 day	14 day
Vehicle control	2.72±0.61	1.84±0.44	3.37±0.69	2.56±0.62
Scopolamine hydrobromide	2.53±0.27	1.19±0.17	1.40±0.78	1.63±0.31
*Brassica oleracea(100)+*Scopolamine (0.4)	3.69±1.11	3.14±0.52	4.15±0.75	5.84±1.45
Brassica oleracea (200)+ Scopolamine (0.4)	3.88±0.76	3.40±0.65	5.29±1.41	6.59±1.54
Piracetam(120)+ Scopolamine (1)	4.54±0.78	3.91±0.52	5.77±1.05	6.80±1.79

Values are expressed as mean ± SEM at n=6; one way ANOVA followed by Dunnett's Test

Biochemical estimation

AChE levels, which is considered an indicator of inhibition of AChE activity in the brain of rat after 7 days of treatment. However, in 14 days of treatment, Piracetam (120 mg / kg po) and *Brassica oleracea* (100 and 200 mg / kg po) significantly reduced (P <0.01) AChE levels, which is considered an indicator of inhibition of the activity of AChE **[Table 3].**

Table 3: Effect of the extract of *Brassica oleracea* on AChE Inhibition Activity

Treatments (mg/kg)	AChE concentrated (μMol/minute/g of tissue)	Inhibition of AChE activity (%)
Control	6.742±0.19	
Scopolamine hydrobromide	12.39±0.35	
*Brassica oleracea (100)+*Scopolamine (0.4)	6.023±0.37	51.38
*Brassica oleracea (200)+*Scopolamine (0.4)	5.207±0.29	57.97
Piracetam(120)+ Scopolamine hydrobromide(0.4)	4.962±0.26	59.09

Values are expressed as mean ± SEM at n=6; one way ANOVA followed by Dunnett's Test

DISCUSSIONS

Alzheimer's disease is a neurogenerative disorder associated with a decrease in cognitive abilities. [12]. Despite high prevalence of AD, the allopathic system of medicine could not provide a satisfactory medicine with minimal side effect. Therefore, this study focuses on the searching of the memory enhancing activity of the *Brassica oleracea* extract in scopolamine induced amnesic rat model. This study suggests that vegetable such as *Brassica oleracea* can possess cognitive enhancing activity in view of its facilitating effect on the retention of spatial memory in the scopolamine induced amnesia . There is a decrease in TL, that is, rats were able to locate the dark area immediately after exposure to the open arm in the EPM paradigm, which is an indicator of cognitive improvement. [13]

In the case of the passive avoidance paradigm, SDL is increased with the *Brassica oleracea* extract. This suggests that the animal retains the memory of the discharge once it has entered the area without discharge. The long-term administration of *Brassica oleracea* extract (14-day administration) showed a pronounced effect on the reversal

of scopolamine induced amnesia in the case of the passive avoidance paradigm compared to the 7-day administration.

It has been investigated and found that cholinergic neuronal systems play an important role in the cognitive deficits associated with AD, aging and neurodegenerative diseases. [14] In our study, Piracetam (120 mg / kg p.o.) and 200mg/kg *Brassica oleracea* extract significantly reduced AChE activity, indicating the stimulatory actions of these drugs in the cholinergic system. Hence, memory improving effect of the *Brassica oleracea* can be attributed to its anti CHE activity. [15]

Thus, it can be used to delay the onset and reduces the severity of Alzheimer's disease. However, further researches should be carried out to explore the possible involvement of other neurotransmitters such as glutamate, GABA and catecholamine, in memory and learning. [16]

CONCLUSION

In the present study, we observed that *Brassica oleracea* reduced elevated acetylcholine (ACh) levels in the brain which ultimately, improved memory (spatial and avoidance) of rats when its extract administered to rats for 7 days and 14 days, respectively. In light of the above, it may be worth exploring the potential of this plant in the management of cognitive dysfunction.

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