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Evaluation of Memory Enhancing Activity of Chloroform Extract of Plumbago zeylanica Linn. by Different Models in Rats

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ABSTRACT

AIM- The aim of the present investigation is to Evaluate Memory Enhancing Activity of Different Extracts of *Plumbago zeylanica* Linn. with Special Reference to Chloroform Extract. **MATERIAL & METHODS-** The dried fruits of *PZ* were extracted by successive solvent extraction method with the help of soxhlet apparatus. The plant material first extracted with petroleum ether then chloroform, ethanol and water. The Morris water maze consists of a circular pool (60 cm in diameter, 26 cm in height), filled with water to the depth of 20 cm and made opaque white color. The pool was divided into four hypothetical quadrants. The elevated plus maze served as the exteroceptive behavioral model (wherein the stimulus existed outside the body) to evaluate learning and memory in mice. The apparatus consisted of two open arms and two covered arms. At the end of experiment, the experimental animals were sacrificed by cervical dislocation and brains were taken out. They were rinsed thoroughly with ice-chilled 0.9% NaCl and weighed. A 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). The post nuclear fraction was obtained by centrifugation of the homogenate at 12000g for 60 min at 4ºC. **RESULTS-** Animals treated with chloroform extract at 100 mg/kg showed an acquisition and retention profile reduction in mean latencies and time spent as 148.53±3.34 and 52.50 ± 1.56 respectively significant as compared to scopolamine control group. Chloroform extract treated groups animals showed significant decrease in transfer latency (s) of acquisition trial as compared to disease control animals, thus suggested marked improvement in memory. The reference standard Piracetam (PC) treated animals showed marked significant decrease in transfer latency (s) of acquisition trial as compared to scopolamine disease control animals. **CONCLUSION-** On the basis of the preliminary screening of the *Plumbago zeylanica* extracts we concluded that chloroform extract significantly improved learning memory in several exteroceptive and interoceptive behavioural models of scopolamine induced memory deficit activity, antioxidant activity and by attenuating the biochemical perturbations caused by cognitive impairments.

KEYWORDS

Memory Enhancing Activity, Different Extracts, *Plumbago zeylanica* Linn., Chloroform Extract, Anti-oxidant Activity

INTRODUCTION

According to the World Health Organization (WHO) major population of some Asian and African countries presently uses herbal medicine for some aspect ofprimary health care as well as for treatment of diseases and disorders. The synthetic drugs are more costly, having narrow margin of safety and more side effects as compared to herbal drugs which have wide margin of safety, cheaper andmild side effects than synthetic drugs. Herbal drugs have enormous therapeutic potential for central nervous system (CNS) activity and peripheral nervous system (PNS) activity (Rahmath *et al*., 2015). During the history of mankind, drugs

acting the CNS have focused on those that bring relief to psychiatric disorders andherbal therapy can still provide support having mental problems that also develop symptoms of anxiety, depression and sleep disturbances etc. Dementia, Parkinson disease (PD), Alzheimer's disease (AD) etc are CNS associated problems which are being addressed by herbal treatment (Bear *et al*., 2007). The hippocampus and amygdala are relevant to the storage of recent memory and emotional behavior. The structure of these areas is highly plastic, particularly in the hippocampus while the amygdala acts as a part of a larger intermediate memory systemthat supports learning and performance before habit consolidation. Report on study of monkeys explained that these areas interact with each other while operate independently. There are various evidences which show that hippocampal synaptic plasticity is dependon β –noradrenergic receptor mediated modulation (Shiksharthi *et al*., 2011). β -noradrenergic receptors play a role in memory consolidation mainly by interaction with other neurotransmitter systems, particularly GABAergic, cholinergic and opioid systems at the site of amygdale (Maria *et al*., 2018).

Natural herbal medicines are now attracting researchers in the development of several formulations due to their past history of traditional uses. Herbs have been used since ancient times in the treatment of several diseases and disorders due to low side effects and higher efficacy. There are various traditional herbal medicines which have used worldwide in the form of crude herbal extracts or herbal formulation in alleviating and curing nervous disorders as a memory enhancer and nervine tonic properties (Ilaria Liguori *et al*., 2018). The aim of present work is to find the memory enhancing effect of *Plumbago zeylanica* (PZ) Linn. by suitable method.

MATERIALS AND METHODS

Collection of Plant Material

The dried roots of *PZ* were purchased from authorized herbal supplier of local market.

Succesive Solvent Extraction

The dried fruits of *PZ* were extracted by successive solvent extraction method with the help of soxhlet apparatus. The plant material first extracted with petroleum ether then chloroform, ethanol and water. The all extracts were dried and percentage yield were calculated. All the extracts were examined for the preliminary phytochemical evaluation.

Acute oral toxicity Studies

The acute toxicity study was carried out in adult female albino rats by “fix dose” method of OECD (Organization for Economic Co-operation and Development) Guideline No.420. Fixed dose method as in Annex 2d: Test procedure with a starting dose of 2000 mg/Kg body weight was adopted. The animals were fasted overnight and next day extracts were suspended in 0.5 % w/v sodium CMC and administered orally at a dose level 2000 mg/kg. Then the animals were observed continuously for three hour for general behavioral, neurological, autonomic profiles and then every 30 min for next three hour and finally for mortality after 24 hour till 14 days. The observations were tabulated according to Irwin’s table. The $1/10^{th}$ & $1/5^{th}$ of the maximum tolerable dose 2000 mg/kg was selected for the present studies (Kalandar Ameer, 2016).

Scopolamine Induced Memory Impairment in Mice

Memory impairment, the most important component of dementia, was induced in mice by intraperitoneal administration of scopolamineand. Scopolamine (3 mg/kg), a muscarinic receptor antagonist, was dissolved in normal saline (0.9 % NaCl) and administered intraperitoneally in a volume of 1 ml/kg body weight. Rats were subjected to behavioural testing 5 min after scopolamine injection (Deb *et al*., 2013).

Morris Water Maze Test

The Morris water maze consists of a circular pool (60 cm in diameter, 26 cm in height), filled with water (26 ± 1oC) to the depth of 20 cm and made opaque white color. The pool was divided into four hypothetical quadrants. An escape platform was placed 1 cm below from the water surface. Four different starting points were placed around the perimeter

of the pool. On each of the five training days all four start points were used once in a pseudorandom sequence. Each animal was subjected to a daily session of four trials per day for rats and three trials per session in case of mice for five consecutive days. Escape latency time (ELT) to reach the hidden platform in water maze was noted as an index of learning. The treatment schedule is as follows (Singh *et al*., 2012).

Group-1 (NC): Normal Control, animals received 0.1% NaCMC solution as a vehicle
Group-2 (DC): Diseased Control, Scopolamine (3 mg/kg i.p)
Group-3: Pet. Ether extracts at the doses of 100 mg/kg of body weight p.o. + scopolamine (3mg/kg i.p)
Group-4: Chloroform extracts at the doses of 100 mg/kg of body weight p.o. + scopolamine (3 mg/kg i.p)
Group-5: Ethanolic extract at the doses of 100 mg/kg of body weight p.o. + scopolamine (3 mg/kg i.p)
Group-6: Water extracts at the doses of 100 mg/kg of body weight p.o. + scopolamine (3 mg/kg i.p)
Group-7 (PC): Positive Control, Piracetam (10 mg/kg p.o) + scopolamine (3 mg/kg i.p)

Elevated plus Maze test:

The elevated plus maze served as the exteroceptive behavioral model (wherein the stimulus existed outside the body) to evaluate learning and memory in mice. The apparatus consisted of two open arms (16 cm x 5 cm) and two covered arms (16 cm x 5 cm x 12 cm). The arms extended from a central platform (5 cm x 5 cm), and maze was elevated to a height of 25 cm from the floor. On the first day, each animal was placed at the end of open arm, facing away from the central platform. Transfer latency (TL) was taken as the time taken by animal to move into one of the covered arm with all its four legs. TL was recorded on the first day. If the animal did not enter into one of the covered arm within 90 s, it was gently pushed into one of the two covered arms and the TL was assigned as 90 s. The animal was allowed to explore the maze for 10 s and then returned to its home cage. Memory retention was examined 24 h after the first day trial on the second day (Indrajeet *et al*., 2010).

Group-1 (NC): Normal Control, animals received 0.1% NaCMC solution as a vehicle
Group-2 (DC): Diseased Control, Scopolamine (3 mg/kg i.p)
Group-3: Pet. Ether extracts at the doses of 100 mg/kg of body weight p.o. + scopolamine (3mg/kg i.p)
Group-4: Chloroform extracts at the doses of 100 mg/kg of body weight p.o. + scopolamine (3 mg/kg i.p)
Group-5: Ethanolic extract at the doses of 100 mg/kg of body weight p.o. + scopolamine (3 mg/kg i.p)
Group-6: Water extracts at the doses of 100 mg/kg of body weight p.o. + scopolamine (3 mg/kg i.p)
Group-7 (PC): Positive Control, Piracetam (100 mg/kg p.o) + scopolamine (3 mg/kg i.p)

Dissection and Homogenization

At the end of experiment, the experimental animals were sacrificed by cervical dislocation and brains were taken out. They were rinsed thoroughly with ice-chilled 0.9% NaCl and weighed. A 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). The post nuclear fraction was obtained by centrifugation (Remi - C-30, Remi Industries Ltd, Mumbai, India) of the homogenate at 12000g for 60 min at 4ºC (Israni *et al*., 2010).

Estimation of markers of oxidative stress

Catalase (CAT): The incubation mixture of brain homogenate contained in a final volume of 2.0ml, 0.1ml of diluted homogenate, 1.0ml of phosphate buffer and 0.4ml of distilled water to which 0.5ml of H2O2 solution was added to initiate the reaction, while the H2O2 solution was left out in control tubes. After incubating for 1 min at 37oC the reaction was stopped by addition of 2 ml of potassium dichromate acetic acid reagent. The samples were kept in boiling water bath for 15 minutes, finally cooled and the absorbance measured at 570 nm against control. The catalase content was calculated by using molar extinction coefficient = 58.03 x 10-3 M -1 cm-1 and the values are expressed as nmoles/mg protein (Beg *et al*., 2009).

Lipid peroxidation (LPO): Briefly, the reaction mixture contained 0.1 ml of brain regions homogenate/ mitochondria (1mg protein), 1.5 ml of 20% acetic acid (pH 3.5), 1.5 ml of 0.8% thiobarbituric acid (0.8% w/v) and 0.2 ml SDS. Following these additions, tubes were mixed and heated at 95 °C for one hour on a water bath

and cooled under tap water before mixing 1 ml of distilled water and 5ml mixture of n-butanol and pyridine (15:1). The mixture was centrifuged at 2200g for 10 min. The amount of MDA/TBARS formed was measured by the absorbance of upper organic layer at a wave length of 532 nm. The results are expressed as nmol MDA/mg protein. The absorbance of the clear pink color supernatant was measured at 532 nm against appropriate blank. The amount of lipid peroxidation was determined by using molar extinction coefficient 1.56 x 105 M - 1 cm-1 and the results were expressed as nmoles MDA/g of protein (Bajpai *et al*., 2010).

Reduced Glutathione (GSH): The assay is based on the principle of Ellman's reaction. The sulfhydryl group of glutathione reacts with DTNB (5, 5'-dithiobis-2-nitrobenzoic acid) and produces a yellow colored 5-thio- 2-nitrobenzoic acid (TNB). Measurement of the absorbance of TNB at 412 nm provides an accurate estimation of glutathione in a sample. Briefly, 0.5 ml of homogenate is mixed with 0.1 ml of 25% TCA to precipitate proteins and centrifuged at 4000 rpm for 5 min. Then, 0.3 ml of the supernatant was mixed with 0.5 ml of 0.1M phosphate buffer (pH 7.4) and 0.2 ml of 10 mM DTNB. This mixture was incubated for 10 min and the absorbance was measured at 412 nm against appropriate blanks. The glutathione content was calculated by using extension coefficient 13.6 x 103 (Srivastav *et al*., 2010).

RESULTS

% Yield of different extracts

The % yield of petroleum ether, chloroform ethanolic and water extracts of dried roots was found 16.5, 7.4, 2.81, 9.2 and 5.8 % respectively.

Qualitative chemical examination of different extracts

On qualitative phytochemical examination of different extracts, the test for carbohydrate was found positive in ethanolic and chloroform extract. Proteins were found in ethanolic, chloroform extract. The steroidal test was positive in petroleum ether extracts. Triterpenoids test was found positive in ethanolic, chloroform extract. Glycosides were present in ethanolic, chloroform extract. No extract was given positive test for presence of alkaloids. Test for flavonoids was found positive in ethanolic extract of. The test of phenolic and tannin compounds was positive in ethanolic, chloroform extracts but highest in ethanolic extract.

Effect of different extracts on the scopolamine induced spatial learning and memory deficit using Morris water maze test

Spatial learning in the water maze was analysed during acquisition trial in term of escape latencies. On contrary, the latencies in the target quadrant exhibited by scopolamine treated group significantly higher to the extent and time spent is significantly lower to the extent respectively as compared to normal control group. The results indicated that control animals learned correctly the platform location than scopolamine treated animals. Animals treated with chloroform extract at 100 mg/kg showed an acquisition and retention profile reduction in mean latencies and time spent as 148.53±3.34 and 52.50 ± 1.56 respectively significant as compared to scopolamine control group.

Table No. 1: Effect of different extracts on the scopolamine induced spatial learning and memory deficit using Morris water maze test

S. No.	Treatment groups	Mean latencies	Time spent in the target quadrant
1	Normal Control	130.71 ± 1.61***	64.23 ± 1.33***

2	Disease Control	196.82 ± 2.40	39.83 ± 2.73
3	Pet Ether Extract	176.53 ± 2.88***	42.50 ± 2.57*
4	Chloroform Extract	148.53± 3.34***	52.50 ± 1.56***
5	Ethanol extract	157.11 ± 2.33***	47.67 ± 2.46***
6	Water Extract	163.25 ± 1.23***	43.00 ± 2.53**
7	Positive Control	135.52 ± 2.82***	56.67 2.45***

Effect of different extracts on the transfer latency in the acquisition (AT) using elevated plus maze in the scopolamine induced amnesiac rats

In a scopolamine treated group animals the transfer latency (s) increased significantly to the extent of Seconds acquisition trial as compared to normal vehicle control animals thus, suggesting marked impairment *of* memory. Chloroform extract treated groups animals showed significant decrease in transfer latency (s) of acquisition trial as compared to disease control animals, thus suggested marked improvement in memory. The reference standard Piracetam (PC) treated animals showed marked significant decrease in transfer latency (s) of acquisition trial as compared to scopolamine disease control animals.

Table No. 2: Effect of different extracts on the transfer latency in the acquisition (AT) using elevated plus maze in the scopolamine induced amnesiac rats

S. No.	**Treatment groups**	**Transfer latency** (s) **in the AT**
1	Normal Control	34.33 ± 2.78***
2	Disease Control	53.67 ± 1.44
3	Pet Ether Extract	36.33 ± 2.87***
4	Chloroform Extract	41.50 ± 1.58***
5	Ethanol extract	47.50 ± 2.22***
6	Water Extract	45.17 ± 2.72***
7	Positive Control	30.83 ± 1.60***

Effect of different extracts on the open arm entry and time spent in the acquisition (AT) using elevated plus maze

In a scopolamine treated group animals significantly decreased time spent and number of entries made in the open arm to the extent of as compared to normal vehicle control group. Chloroform extract treated groups animals showed significant increase in time spent and number of entries made in the open arm to the extent of as compared to disease control group respectively, thus suggested marked improvement in memory. The reference standard Piracetam treated animals showed marked significant increase in time spent and number of entries made in the open arm to the extent as compared to disease control group animals.

Table No. 3: Effect of different extracts on the open arm entry and time spent in the acquisition (AT) using elevated plus maze in the scopolamine induced amnesiac rats

S. No.	Treatment groups	Mean time spent (s) in the open arm	No. of entries made in the open arm
1	Normal Control	14.17 ± 1.56***	8.21 ± 1.31***
2	Disease Control	5.250 ± 1.82	2.33 ± 1.16
3	Pet Ether Extract	12.28 ± 0.42***	6.67 ± 0.46***
4	Chloroform Extract	6.83 ± 1.73**	4.33 ± 0.33***
5	Ethanol extract	8.83 ± 0.31***	5.50 ± 0.26***
6	Water Extract	7.83 ± 0.33***	3.41 ± 0.84***
7	Positive Control	13.33 ± 1.35***	7.17 ± 1.73***

Effect of different extracts on the markers of oxidative stress Catalase (CAT) level

In a scopolamine treated group animals the brain tissue catalase level significantly decreased as compared to normal vehicle control group. Chloroform extract treated groups animals showed significant increase in brain tissue catalase level as compared to disease control animals, thus suggested marked improvement in memory. The reference standard Piracetam (PC) treated animals showed marked significant increase in brain tissue catalase level as compared to disease control animals.

Table No. 4: Effect of extracts on the markers of oxidative stress Catalase (CAT) level

S. No.	Treatment groups	Catalase (CAT) level (nmoles/mg protein)
1	Normal Control	64.24 ± 2.85***
2	Disease Control	35.13 ± 2.41
3	Pet Ether Extract	69.11 ± 3.49***
4	Chloroform Extract	44.64 ± 2.83***
5	Ethanol extract	57.28 ± 1.75***
6	Water Extract	38.73 ± 2.86**
7	Positive Control	73.12 ± 3.70***

Effect of extracts on the markers of oxidative stress Lipid peroxidation (MDA) level

In a scopolamine treated group animals the brain tissue Lipid peroxidation (MDA) level significantly increased m as compared to normal vehicle control group. Chloroform extract treated group animals showed significant decreased in brain tissue Lipid peroxidation (MDA) level as compared to disease control animals, thus suggested marked improvement in memory. The reference standard Piracetam (PC) treated animals showed

marked significant decreased in brain tissue Lipid peroxidation (MDA) level as compared to disease control animals.

Table No. 5: Effect of extracts on the markers of oxidative stress Lipid peroxidation level

S. No.	Treatment groups	MDA level (nm/gm)
1	Normal Control	209.14 ± 2.66
2	Disease Control	609.62 ± 3.10
3	Pet Ether Extract	236.45 ± 3.49**
4	Chloroform Extract	280.32 ± 2.17***
5	Ethanol extract	394.33 ± 1.12***
6	Water Extract	550.81 ± 3.67**
7	Positive Control	210.72 ± 3.83***

Effect of extracts on the markers of oxidative stress Reduced Glutathione (GSH) level

In a scopolamine treated group animals the brain tissue Reduced glutathione level significantly decreased as compared to normal vehicle control group. Chloroform treated groups animals showed significant increase in brain tissue Reduced glutathione level as compared to disease control animals, thus suggested marked improvement in memory.

The reference standard Piracetam (PC) treated animals showed marked significant increase in brain tissue Reduced glutathione level as compared to disease control animals.

Chloroform extract treated animals showed significant highest improvement in antioxidant Reduced glutathione level as compared to other extract treated groups.

Table No. 6: Effect of extracts on the markers of oxidative stress Reduced Glutathione level

S. No.	Treatment groups	Reduced glutathione Level (nm/mg)
1	Normal Control	1.58± 0.95***
2	Disease Control	0.850 ± 0.30
3	Pet Ether Extract	1.42 ± 0.67***
4	Chloroform Extract	0.99± 0.72**
5	Ethanol extract	1.23 ± 0.87***
6	Water Extract	0.95 ± 0.21*
7	Positive Control	1.57 ± 0.29***

DISCUSSION

Learning is defined as acquisition of information and skills and subsequent retention of this information is called as memory. In Ayurveda, there are three aspects of mental ability eg. Dhi (process of acquisition/learning), Dhuti (process of retention) and Smriti (process of recall). Any disturbance in these aspects resulted in the loss of mental ability. Memory is the process by which organisms are able to record their experiences and retain them over short or long periods of time and recall the same at a later time when needed. Memory plays a vital role in human life, as without it, one cannot lead a normal life (Sanchti *et al.,* 2010).

Poor memory, lower retention and slow recall are common problems in today's stressful and competitive world. Age, stress, emotions are conditions that may led to memory loss, amnesia, anxiety, high blood pressure, dementia, to more ominous threat like schizophrenia and Alzheimer's diseases (Reddy *et al.,* 2009).

Some plant extracts which occur as a complex mixture of components, such as *Plumbago* species extract, have demonstrated relevant biological activities in relation to metabolism, cardiovascular, diabetes, disinfection, antimicrobial and antioxidant potential, but the compounds responsible for the observed effects or the mechanisms of action have not been well characterized. Therefore, scopolamine was used to study effect of *Plumbago zeylanica* on memory impairment function (Prasad *et al.,* 2009).

The Morris water maze is a behavioral procedure widely used in behavioral neuroscience to study spatial learning and memory. In this paradigm, animal is placed into a small pool of water which contains an escape platform hidden below the water surface. Visual cues, such as colored shapes, are placed around the pool in plain sight of the animal. Escape from the water reinforces a desire to quickly find the platform, and on subsequent trials subjects are able to locate the platform increasingly rapidly. This improvement in behavioral performance occurs presumably as a result of learning and memory for where the hidden platform is located relative to the conspicuous visual cues.

Scopolamine was administered 5 min before the acquisition trial to induce memory impairment and retention was tested. Administration of scopolamine caused memory impairment in rats as indicated by significant increase in transfer latency time in elevated plus maze (Cherian *et al.,* 2009).

Based on the aversive stimuli behavioural models for studying the neurobiology of learning and memory can be broadly classified into two types exteroceptive (the aversive stimuli for learning and memory originating outside the body) or interoceptive (the aversive stimuli for learning and memory originating inside the body). Most of the currently used paradigms for learning and memory can be conveniently discussed under two of the following behavioural tasks: behaviour on the mazes and behaviour on avoidance chambers (eg. active and passive avoidance task paradigms).

In Morris water maze study we directly examined the effect of *Plumbago zeylanica* extracts on mean latencies during the acquisition trial and time spent in the target quadrant. Scopolamine treated disease control group significantly increased mean latencies to the extent as compared to normal vehicle control group. Animals orally treated with *T. chebula* fruit extracts and fraction significant decreased the mean latencies of the acquisition trial as compared to scopolamine disease control group. The Significant decrease in transfer latency by chloroform extract as compared to scopolamine disease control group suggests improved beneficial effect on learning and memory process.

Elevated plus maze is the traditional tool in assessing learning and memory performance in laboratory animals. Originally designed to evaluate the antianxiety agents, and recently been extended to measure the cognitive performance notably to evaluate spatial long term memory. Transfer latency as an index of learning is measured indicating time taken by the animal to move from the open arm to one of the covered arms with all its four legs. Significant reduction in the transfer latency during acquisition trial indicated improvement in the learning and memory process (Cherian *et al.,* 2009).

Chloroform extract of *Plumbago zeylanica* with respective doses of 100 mg/kg showed reduction in transfer latency when compared to disease control group. Significant reduction in the transfer latency by chloroform extract as compared to disease control suggesting beneficial effect on learning and memory process (Cherian *et al.,* 2009).

The preliminary phytochemical studies revealed the presence of protein, glycoside, flavonoid, Triterpenoids and phenolic & tannin compound which is in congruence with the documented literature.

Reactive species produced in the cell during normal cellular metabolism can chemically react with cellular biomolecules such as nucleic acids, proteins, and lipids, thereby causing their oxidative modifications leading to alterations in their compositions and potential damage to their cellular activities. Also with increasing evidence suggests that reactive oxygen species (ROS) such as catalase(CAT), malondialdehyde (MDA), reduced glutathione hydroxyl (GSH), nitric oxide and peroxide act as necessary signaling molecules in the process underlying cognition. Moreover ROS have been shown to be necessary in molecular process underlying signal transduction, synaptic plasticity and memory formation (Trifiro *et al.,* 2009).

CONCLUSION

Lack of satisfactory treatment of the cognitive deficits usually accompanying stress, depression, anxiety, ageing, and associated mental problems presents a constant challenge for Psychopharmacological research. On the basis of the preliminary screening of the *Plumbago zeylanica* extracts we concluded that chloroform extracts significantly improved learning memory in several exteroceptive and interoceptive behavioural models of scopolamine induced memory deficit activity, antioxidant activity and by attenuating the biochemical perturbations caused by cognitive impairments.

Plumbago zeylanica chloroform extract & standards share number of promnesic physiological actions that are potentially beneficial in groups showing cognitive decline and it seems reasonable to suggest that they could be considered as a strategy to prevent or slow down the development of decrease in memory diseases such as Amnesia, dementia and Alzheimer's disease at an early stage.

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