ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

PHARMACOLOGICAL EVALUATION OF *ANETHUM GRAVEOLENS* (DILL) FRUIT EXTRACTS IN ANIMAL MODEL OF MEMORY ENHANCEMENT

Chakresh Patley[1], Rakesh Sagar[2], Mohan Lal Kori*[1]

[1] *Vedica college of B. Pharmacy, RKDF University, Gandhi Nagar, Bhopal, MP, India*

[2] *Shri Govindram Seksaria Institute of Technology and Science, Indore, MP, India*

**Corresponding author: mlkori.research@gmail.com*

ABSTRACT

Phytochemical investigations of plant develop a valuable phenomenon for scientists for new innovations and sophisticated drug discoveries. These chemical materials produced related to plants having medicinal values and that shows various biological properties. The examples are glycosides, alkaloids, tannins, flavonoids and phenolic compounds. The investigation of chemical constituents is useful for the discovery of therapeutic agents, but also for disclosing new resources of such chemicals. The proposed work was done on the chloroform, ethyl acetate and methanol extract of *Anethum graveolens*. The memory-enhancing effects were evaluated by studies *i.e.* anti-inflammatory activity, antioxidant activity (Elevated plus Maze Model), acetylcholine Esterase inhibitory activity (Elevated plus Maze Model), open-field test, morris water maze test and step-down test on normal and aged mice. The tests showed the impaired spatial memory of the aged mice was partly reversed by ethyl acetate extract (100 mg/kg; $P < 0.05$) as compared with the aged control mice. In step-down tests, the nonspatial memory of the aged mice was improved by ethyl acetate extract (100 mg/kg; $P < 0.05$). Additionally, ethyl acetate extract of plant could inhibit acetyl cholinesterase (AChE), and also showed anti-inflammatory and anti-oxidant activity in the brain tissue of the aged mice. The results showed that ethyl acetate extract (100 mg/kg; $P < 0.05$) improved memory functions of the aged mice probably via its antioxidant and anti-inflammatory properties. Thus the treatment on animals with ethyl acetate extract did not cause significant change in the cortex of brain and have no side effect. Thus the proposed work is showed that plant extract could be used to treat cognitive dysfunction and further could be used for memory enhancement.

Keywords: Phytochemical, Phenolic; Flavonoid, Antioxidant, Anti-inflammatory.

1. INTRODUCTION

The human brain is the well-designed and composite structure ever created by creature. It consist of some ten billion neurons and supportive cells in structure. There is no other organized structure can match with the complexicity and processing power of human brain. It controls practically all life systems while concurrently produce the thoughts, dreams and feelings that describe us and shape our observation of reality. The brain is the biological organ responsible for thinking, memory, reasoning, and language. The memories are stored in different parts of the brain. Many scientists believe that the entire brain is involved with memory. However, since Lashley's research, [1] other scientists have been able to look more closely at the brain and memory. They have argued that memory is located in specific parts of the brain, and specific neurons can be recognized for their involvement in forming memories. The main parts of the brain involved with memory are the amygdala, the hippocampus, the cerebellum, and the prefrontal cortex [1]. Memories are primary basic part of human identity which makes human as unique creature of earth. Memories also control the human behavior at every time by reminding its past actions and effects. If any human have dislocate ability to form recollections thus existence of that being turn out to be very hard and isolating from social environment [2]. An individual can record sensory stimulus, actions, information, etc preserve for dumpy or long stage of moment and while desired, remind the matching at shortly owed to memory. Poor Memory is a lower preservation and slow recall. Now days it is an ordinary problem due to stressful and competitive life. Factors such as lack of sleep, thyroid problems, alcohol abuse, stress, anxiety or depression and some medication can cause loss of memory. There are two kind of memory, One is temporary or short term memory is the

capacity to store a small amount of information in the mind and keep it readily available for a short period of time [3]. The word "Cognition" is a fancy word that mental health professionals use to describe the wide range of brain-based behaviors that we rely on every day. Cognition encompasses different skills, including perception (taking in information from our sensory organs), memory, learning, judgment, abstract reasoning (thinking about things that aren't directly in front of us), and problem solving using language, and planning. Cognition related to the mental action or process of acquiring knowledge and understanding through thought, experience, and the senses. It is in essence, the ability to perceive and react, process and understand, store and retrieve information, make decisions and produce appropriate responses. All these abilities are inter-related and work together to function properly by any human in environment. Cognitive skills are not similar as academic skills because academic skills include different subject's knowledge such as literature, math, history and science. Cognitive skills is mental capabilities required to learn subject matter and it helps function in daily life. Cognitive skills are the fundamental skills that helps in think, read, understand, remember, plan and organize. The cognitive dysfunction is also recognized as brain fog in various journals [3]. The brain fog is the loss of intellectual role like judgment, recollection, and logic with adequate brutality to make smooth every day running. Cognitive dysfunction has indication like danger with vocal recollect, crucial reckoning, and attention. As per the guidelines in the Marshall Pathogenesis [3], cognitive dysfunction might be owing to germs. Cognitive dysfunction has come into view in strict shape in diseases such as Alzheimer's diseases and frequently linked with chronic exhaustion syndrome. A mirror image study of cognitive function in chronic fatigue syndrome was observed as the effects of unexpected illness beginning [4]. It is also in attendance in with manifold sclerosis patients with indication like estimation, imaging, and risk feature [5], depression [4], fibromyalgia [6]. Cognitive dysfunction symptoms are associated with many others diseases. It may momentarily enhance during periods of immunopathology. Some studies states that sick women face cognitive dysfunction more often and severely than their male counterparts [7]. Most common cause of cognitive problems is the illnesses themselves that cause most of the cognitive dysfunctioning. It is myth about cognitive dysfunction for many years that it is secondary to other symptoms, like psychosis, lack of motivation, or unstable mood. It is a primary symptom of schizophrenia and many other psychotic disorders. Cognitive problems may be apparent when other symptoms are controlled or not present.

Besides, research has revealed that parts of the brain that are used for specific cognitive skills, frequently do not function normally in people with schizophrenia and certain other disorders. This revealed that mental illness affects the brain functions, and causes cognitive problems. There are many myths about mental illness and cognitive dysfunction [8].

There are various techniques have been developed for memory enhancement and prevention from age-related memory loss. One of these strategies is the use of some medicinal plants with memory enhancing abilities. Dill is the Satahva (Fruit); comprises of the dried fruits of *Anethum graveolens* (AG) Family Apiaceae,that is traditionally used by Indian Ayurvedic system for memory enhancing properties [9]. AG is an annual vertical plant with 50-150 cm stem belonging to the Apiaceae family. The fruits of AG are brown, flat, tiny, and oval in shape. This plant is cultivated in Mediterranean countries, in Asia, and in Europe. AG contains 36% carbohydrates, 15.68% proteins, 14.80% fiber, 9.8% ash, and 8.39% moisture as well as essential oils, fatty oil, minerals, and vitamins [10]. AG is used in the traditional herbal medicine for the management and prevention of digestive disease, breath problem, motivation of lactation, and also reduction of cholesterol and glucose. Currently, many studies have established these properties; also, AG is recently known as anticancer, antimicrobial, antigastric irritation, anti-inflammatory, and antioxidant agent [10]. In this respect, dill is produced as a hypolipidemic drug (*Anethum* tablet) in Iran which consists of *Anethum graveolens* (68%), *Cichorium intybus* (5%), *Fumaria parviflora* (5%), and lime (Citrus aurantifolia) (4%) [11].

2. MATERIAL AND METHODS

All the chemicals and solvents were of analytical grade (AR Grade) and were purchased from Sigma Aldrich, Ranbaxy fine chemicals Ltd., LOBA chemicals Ltd., S.D. fine chemicals Ltd., Spectrochem chemicals. All the solvents used for analysis were purchased from JT Baker and Fischer scientific Ltd.

2.1. Collection and authentication of plant material

The selected plant material *Anethum graveolens* Fruit was purchased from local market of Bhopal, M. P., India, and authenticated by botanist, RKDF University, Bhopal.

Voucher specimens are submitted to RKDF University, Bhopal, M. P.

2.2. Macroscopic studies

The selected crude drugs were subjected to studies organoleptic characters *i.e.,* color, odour, appearance, taste, texture etc.

2.3. Physicochemical evaluation

Physiochemical qualities, for example, ashs qualities and extractive qualities were evaluated for plant material as per WHO guidelines; quality control techniques for plant materials. The parameters *i.e.* ash values (total ash, acid insoluble ash and water soluble ash), extractive values, loss on drying and pH were determined for *Anethum graveolens* Linn. fruit.

2.4. Extraction process of drug

Extraction includes partition of bioactive segment of the plant tissues from the latent moiety by utilizing specific solvents in standard extraction procedure. The plant herbs were extracted successively with chloroform, ethyl acetate and methanol using soxhlet extraction. The dried fruits of *Anethum graveolens* Linn. were coarsely powdered. Fruit powder (200g) were stuffed in soxhlet apparatus and extracted with chloroform till finish of extraction. The acquired extract was dried under reduced pressure utilizing rotary evaporator to get chloroform extract. The drug powder was then extracted with ethyl acetate to acquire ethyl acetate extract. The drug powder was again extracted with methanol then acquired extract was dried under reduced pressure utilizing rotary evaporator to get methanol extract.

2.5. Preliminary phytochemical analysis of extracts

Qualitative test act as phytochemical examination of any plant species, which is a vital procedure as it give the data about different constituents present and furthermore gives further possibilities of the specific plant species in its future research examinations. The extract acquired by successive extraction were exposed to different tests to recognize the presence of phytoconstituents *i.e.* presence of alkaloid, carbohydrates, glycoside, phytosterols and triterpenoids, protein and amino acids, phenolic and tannins, flavonoids, oils and fats, saponins [11,12].

2.6. *In-vivo* screening for memory enhancement activity

2.6.1. Animals

The *in-vivo* animal experimentation was performed on Swiss albino mice weight. These mice having 20-25g or albino rats weight between 110-150 g were used for various experiments. The animals were kept to animal quarters previous to testing at temperature of 25±2°C and 50±5% with relative humidity in polypropylene cages at a 12 h light/dark cycle and allowable free of charge entrance to food and water. The experiments were achieved by subsequent rules and system of CPCSEA (Committee for the purpose of Control and Supervision on Experimental Animals) approved by the IAEC IAEC/VCP/2019/04 (Institutional Animal Ethical Committee), RKDF University, Bhopal.

2.6.2. Memory enhancement activity [14-16]

The plants drug Dill; *Anethum graveolens* Linn. (Fruit) extract were evaluated for memory enhancement potentials using the following *in-vivo* models. The various extracts *i.e.* chloroform, ethyl acetate and methanol extract of plant were used for further memory enhancement activity study according to procedures described in subsequesnt sections.

2.6.3. Anti-inflammatory activity

The adult male mice (20-35 g) were taken for the anti-inflammatory experimentation. Adult male wistar rats (150-200 g) were employed to learning the anti-inflammatory movement. The animals were housted in standard laboratory cages in a room where ambient temperature was 25±5°C and a 12h light. The experimentation of anti-inflammatory activity was performed by carrageenan-induced rat paw oedema method [12-13]**.** The Male wistar rats (150-200 g) were arbitrarily dispersed into three groups of five animals each. The first group acted as a control, second group acted as the standard (containing as aceclofenac sodium 10 mg/kg, i.p). The third group of animals received 100 mg/kg, body weight of chloroform extract ChAG, fourth group received 100 mg/kg, body weight of Ethyl Acetate extract EAAG, fifth group received 100 mg/kg, body weight of Methanol extract respectively. After 1 h, 0.1 ml of 1% w/v suspension of carrageenan was injected into the sub-plantar region of the right hind paw to all the three groups. The paw volumes were calculated using plethysmometer (UGO Basile, 7140 Italy) each hour till 3 h after carrageenan injection. The mean amount increase in paw volumes were noted, thus oedema volumes in control (Vc) and in groups treated with test compounds (Vt) were calculated. The percentage inhibition was calculated by using the formula [14].

Percentage of inhibition = 100 (1-Vt / Vc)

Where, Vc= Edema volume in control and Vt= Edema volume in test / standard compound

The results are expressed as mean ± SEM. The statistical analysis was performed by analysis of variance (ANOVA) test.

2.6.4. Antioxidant activity (Superoxide scavenging)

Superoxide scavenging was carried out using the alkaline dimethyl sulfoxide (DMSO) method [14]. Solid potassium superoxide was allowed to stand in contact with dry DMSO for at least 24 hrs and the solution was filtered immediately before use; the filtrate (200 µl) was added to 2.8 ml of aquous solution containing nitroblue tetrazolium (56 µM), EDTA (10 µM) and potassium phosphate buffer (10 µM, pH 7.4). Test solutions at different concentrations (5-100 µg/ml) were added and absorbances were recorded at 560 nm against the control.

2.6.5. Acetylcholine esterase inhibitory activity

Acetylcholinesterase activity was estimated by using an artificial substrate, acetylthiocholine (ATC). In the medium, thiocholine released due to the cleavage of ATC by AChE is allowed to react with the -SH reagent 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), which is reduced to a yellow colored anion called thionitrobenzoic acid, measurable at the wave length 412 nm. The extinction coefficient of the thionitro benzoic acid is 1.36×104/ molar/ cm. The absorption of thionitro benzoic acid is detected by using a UV spectrophotometer to identify direct estimation of the AChE activity [15-16]. The investigation was start to cut and open the brain and immediately transferred brain into the chilled phosphate buffer solution. Hippocampus and cerebrum were disconnected from the frontal cortex part. A part was weighed and transferred to the glass tube of potter Elvehjem homogenizer and added10 volumes of 0.9 % sodium chloride solution and homogenized in an ice bath. Homogenate was centrifuged at 3000 rpm for 10 min. A 0.5 ml cloudy supernatant was pippetted out into 25 ml volumetric flask and made the volume with freshly prepared solution number 2. Four (4) ml portions were pipetted out into two test tubes (4ml each) and 2 drops of solution number 4 into one of the test tube and 1 ml of substrate solution 3 into both test tubes were added. Both the tubes were incubated for 10 min at 30°C. The solution in the tube containing eserine was used for zeroing the instrument.

Computation of result:

The enzyme activity is considered with the following procedure:

R = 5.74x 10-4 x A / CO

Where, R = Rate in moles of substrate hydrolyzed/ minute/gm tissue

A = Change in absorbance / min

CO = Original concent rat ion of the t issue (mg/ml).

2.6.6. Open-Field test

Mice were divided into 6 groups for open-field test, where the first group of animals as normal control group and the second group of animals as aged control group. The third group of animals received galantamine (3 mg/kg). The fourth group of animals received 100 mg/kg, body weight of chloroform extract. The fifth group of animals received 100 mg/kg, body weight of Ethyl Acetate extract. The sixth group of animals received 100 mg/kg, body weight of Methanol extract respectively for a period of 4 weeks before behavioral measurement was assessed. Locomotive activities of mice were tested in open-field on the first day of behavioral test. The effect of plant extract on mice locomotor activities was evaluated automatically using an open-field computer-aided controlling system as described in the literatures [17-18]. The apparatus consists of four metal tanks (30 cm in diameter and 40 cm in height) with a video camera fixed at the top, and the apparatus was illuminated by a light source of 120 Lux on the ceiling. Experiments were performed in a quiet room; four mice were tested simultaneously. Thirty minutes after drug administration, each mouse was placed at the center of the metal tank and allowed to explore freely for 5 min. Then, the distance traveled by mouse was measured for 10 min, which was recorded to evaluate the locomotive activity of the mouse.

2.6.7. Morris water maze test

Morris water maze tests were carried out on day 2 to day 8 and day 23 to day 25 [19]; step-down passive avoidance tests [20] were valued on day 15 to day 16 and day 28, respectively of previously selected group of animals. The apparatus used for the acquisition trials in moris water maze test is a circular water pool (100 cm in diameter and 40 cm in height) with constant clues external to the maze for spatial orientation of the mice. The water was made opaque by adding black ink to prevent animals from seeing the submerged platform. The water temperature was kept at 24-26°C during the whole experiment. An invisible platform (6 cm in diameter and 15 cm in height) providing the only escape from water was placed 1.5 cm below the water surface. The pool was divided into four quadrants by a computerized tracking and image analyzer

system. Two principal axes of the maze intersect perpendicularly to one another to create an imaginary "+." The end of each line demarcates one of the four cardinal points: north (N), south (S), west (W), and east (E). In the acquisition phase, mice were placed in the pool containing platform to adapt to the environment before training. Then mice were subjected to two trials each day for 6 days to find the submerged platform that was located in the center of the SE quadrant of the pool and remained at the same position throughout the whole experiment. Two-day training of four trials contributed to a session. For each trial, the mouse was placed for 15 sec on the platform for learning; then, it was gently released into the pool facing the wall. Four different release points (NE, SE, SW, and NW) were varied randomly for each session. Animals were given a maximum of 60 sec to find the platform. If the mouse failed to find the platform within 60 sec, it was gently guided to the platform and stayed there for 10 sec, and its escape latency was recorded as 60 sec. If an animal found the platform within 60 sec, it was allowed to remain there for 10 sec and was then placed into a cage until next trial. After completion of daily training, the animals were returned to their cages for rest. Escape rate, escape latency, and swimming speed were collected to evaluate the ability of learning and memory function of mice. On the 8th day of behavioral measurement, the spatial probe trials were tested. The platform was removed, and each mouse was placed into the water on the opposite side of the SE quadrant. They were allowed to swim freely for 120s. The crossing numbers over the position at which the platform had been located, the swimming time, and the swimming distance spent in the target quadrant were recorded as measures for spatial memory. Two weeks after Morris water maze tests, memory retention tests were given. Neither the platform nor the starting point was fixed; mice were released in the opposite quadrant. This training had been performed 2 times each day for 3 days. The average of two trials during a day was determined as escape latency for the purpose of evaluating memory retention abilities of mice.

2.6.8. Step-Down Test

The effect of plant extract on mice locomotor activities was evaluated. Step-down passive avoidance tests [20] were valued on day 15 to day 16 and day 28, respectively. Step-down passive avoidance tests were carried out in a chamber to evaluate the effects of plant extract. The plant extract were extract out in different medium *i.e.* chloroform, ethyl acetate and methanol on learning and memory function of the aged mice. The floor of the chamber consisted of copper rods and a well-insulated platform made of rubber in one corner of the chamber. The animals were placed in the chamber for 3 min adaptation at the beginning of training. After adaptation, mice were placed on the floor and received an immediate mild electrical shock for 5 minutes (25 V). To avoid the shock, mice displayed an instinctive reaction to jump back onto the platform. The latency to step down on the grid with all four paws was measured. The time on the safe zone (on the platform) and time on the error zone (on the grid) were recorded within 4 min. The tests consisted of three phases of acquisition, consolidation, and retrieval, which were carried out on day 15, day 16, and day 28, respectively.

3. RESULTS AND DISCUSSION

3.1. Pharmacognostic and phytochemical study

A systematic approach is necessary in pharmacognostic study, which helps in confirmation and determination of identity, purity and quality of a crude drug. *Anethum graveolens* Linn. (Fruit) are in oval shape, dark brown colour, aromatic odour and having warm taste. The results of organoleptic studies are presented in table 1 and fig. 1.

Table 1: Organoleptic identification of plants *Anethum graveolens* fruit

S. No.	Parameters	Observation
1	Shape	Oval
2	Size	4 mm long
4	Odour	aromatic
5	Taste	Warm, slightly sharp
6	Colour	Dark brown
7	Foreign organic matter	No adulterants have been found

The physicochemical parameter such as Ash values (Total ash, Acid insoluble ash and Water soluble ash), Extractive values, Loss on drying and pH of all selected plant drugs were performed. Ash values of crude drug provide an idea about the inorganic composition or earthy matter and other impurities present in drug. The extractive values are mainly useful for the determination of adulterated or exhausted drug. All parameters of selected drugs found within the limit as per API (table 2 - 3). Extracts (chloroform, ethyl acetate, and methanol extract) of selected plant drugs obtained by continuous soxhlet were subjected to qualitative phytochemical tests

to identify the presence of secondary metabolite (*viz.*, alkaloids, glycosides, tannins, flavonoids, sterols, fats, oils, phenols and saponins) present in them. Preliminary phytochemical screening of *Anethum gravelosum* showed the presence of triterpenes in chloroform extract; triterpenes, tannins and flavanoids in ethyl acetate extract; carbohydrate, tannin flavanoid, protein and saponin in methanol extract (table 4).

Table 2: Physicochemical parameters of *Anethum graveolens Linn.* Fruit

S. No.	Parameter	Values (% w/w)
1	Total ash	9.5
2	Water soluble ash	5.03
3	Acid insoluble ash	1.42
4	Moisture content	7.86
5	Foreign organic matter determination	0.9

Table 3: Solvent extractive values (%w/w) of *Anethum graveolens Linn.* Fruit

S. No.	Name of extract	Solvent extractive values (%w/w)
1	Chloroform Extract	4.34 % w/w
2	Ethyl acetate Extract	4.98 % w/w
3	Methanol Extract	11.06 % w/w

3.2. *In-vivo* pharmacological screening for memory enhancement activity

3.2.1. Anti-inflammatory activity

The result of extract of herbs against carrageenan-induced paw oedema is shown in table 5 and fig. 2. All plant extract (100 mg/kg, i.p.) gave significant ($P < 0.01$) reduction on rat paw oedema at predetermined time intervals. The methanolic extract of dill showed maximum inhibition of 54.58 % at the dose of 100 mg/kg after 2 h of drug treatment in carrageenan-induced paw oedema whereas the standard drug showed 56.72 % of inhibition.

Table 4: Phytochemical analysis of *Anethum gravelosum* extracts

S. No.	Phytochemical	Indication test	Chloroform extract	Ethyl acetate extract	Methanol extract
1	Alkaloid	Dragendorff test	-	-	-
2	Napthoquinon	Juglone test	-	-	-
2	Steroid	Salkowaski test	-	-	-
3	Carbohydrat	Molish test	-	-	+
4	Triterpene	Vanillin-sulphuric acid test	+	+	-
5	Tannin	Ferric chloride test	-	+	+
6	Glycosid	Keller-killani test	-	-	-
7	Protein	Biuret test	-	-	+
8	Flavonoid	Shinoda Test	-	+	+
9	Saponin	Lead acetate test	-	+	+

Where + is Present and - is Absent

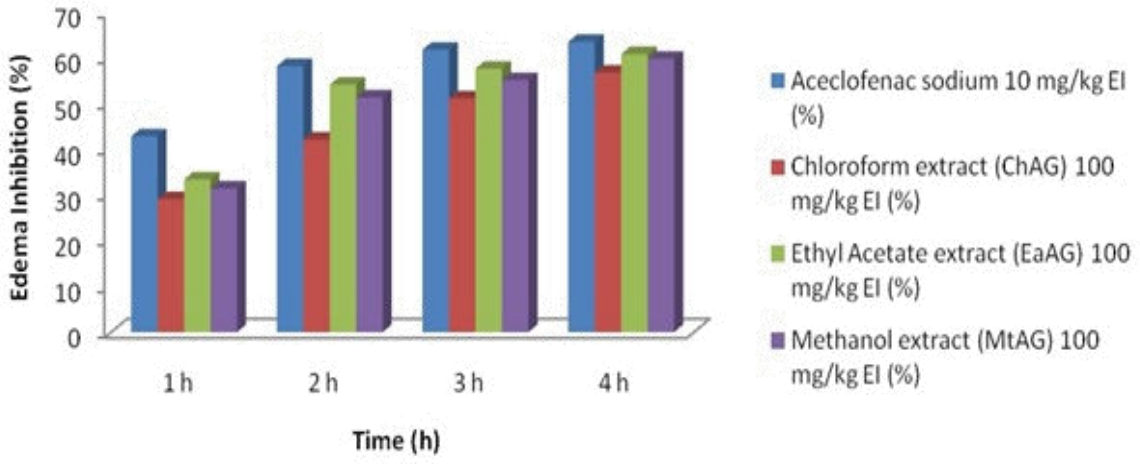

Fig. 1: *In-Vivo* antiinflammatory activity of plant extract

Carrageenan-induced paw oedema was applied as a prototype of exudative phase of acute inflammation during inflammation evaluation. Inflammatory stimuli microbes, chemicals and necrosed cells activate the different mediator systems through a common trigger mechanism. The development of carageenan induced oedema is believed to be biphasic. The early phase is attributed to the release of histamine and serotonin and the delayed phase is sustained by the leucotrienes and prostaglandins. Flavonoids and tannins are reported to inhibit PG synthesis. Most of the non steroidal anti-inflammatory drugs (NSAIDs) have well balanced anti-inflammatory and ulcerogenic activities, which are considered to be due to PG synthetase inhibitor activity.

3.2.2. *Anti-oxidant activity by inhibition of DPPH radical*

The potential decrease in the concentration of DPPH radial due to scavenging property of extract in Dill; Satahva; *Anethum graveolens* Linn. (Fruit) extract and BHT showed significant free radical scavenging activity. The ethyl acetate extracts of plant 77.24% and 87.03% respectively at 100µg/ml (table 6 and fig. 3 - 4).

Table 5: *In-vivo* Antiinflammatory activity

Treatment (Plant extract)	Dose	1 h		2 h		3 h		4 h	
		EV (ml)	EI (%)	EV (ml)	EI (%)	EV (ml)	EI (%)	EV (ml)	EI (%)
Control	-	1.99±0.21	-	1.83±0.33	-	1.81±0.24	-	1.79±0.11	-
Aceclofenac sodium	10 mg/kg	1.11±0.14	42.78	0.92±0.70	58.12	0.86±0.21	61.71*	0.81±0.12	63.41
Chloroform extract (ChAG)	100 mg/kg	1.41±0.01	29.07	1.11±0.11	42.07	0.99±0.07	51.08*	0.94±0.15	56.75
Ethyl Acetate extract (EaAG)	100 mg/kg	1.33±0.21	33.37	0.97±0.14	54.07	0.93±0.47	57.58*	0.88±0.15	60.75
Methanol extract (MtAG)	100 mg/kg	1.38±0.11	31.37	0.99±0.11	51.27	0.95±0.17	55.08*	0.91±0.15	59.75

*Values are mean±SEM, (n=5), *P<0.01; EV=Edema Volume, EI=Edema Inhibition*

Table 6: *In-vivo* Antioxidant activity animal study

Plant extract	Concentration (µg/ml)	DPPH free radical inhibition	Nitric oxide
Chloroform extract (ChAG)	5	4.03±0.13	3.81±0.11
	10	11.41±0.12	7.91±0.13
	25	22.24±0.11	11.08±0.02
	50	38.08±0.01	28.14±0.21
	100	63.16±0.12	54.31±0.21
Ethyl Acetate extract (EaAG)	5	5.28±0.13	5.18±0.21
	10	14.21±0.13	9.21±0.13
	25	29.14±0.18	14.23±0.22
	50	42.88±0.14	31.82±0.11
	100	67.26±0.19	57.21±0.11
Methanol extract (MtAG)	5	4.13±0.03	4.11±0.01
	10	12.01±0.03	8.01±0.03
	25	23.04±0.08	12.03±0.12
	50	39.08±0.11	29.12±0.01
	100	65.06±0.11	55.11±0.01

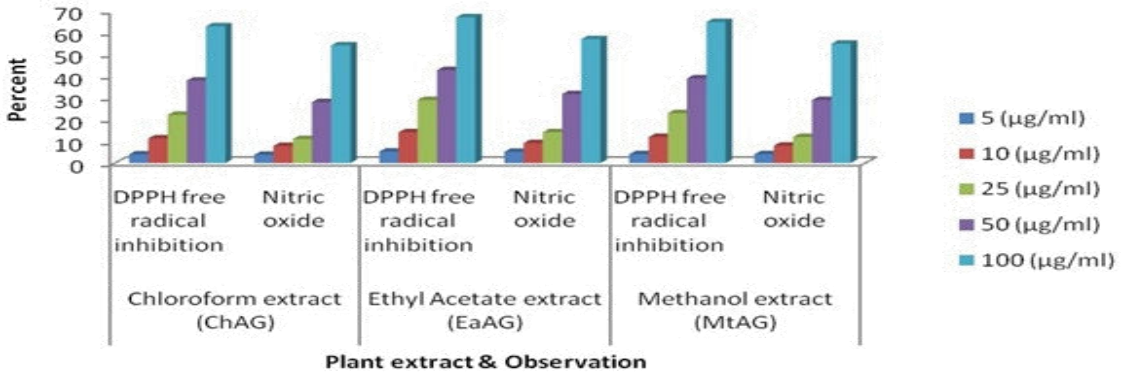

Fig. 2: *In-vivo* Antioxidant activity animal study of plant extract

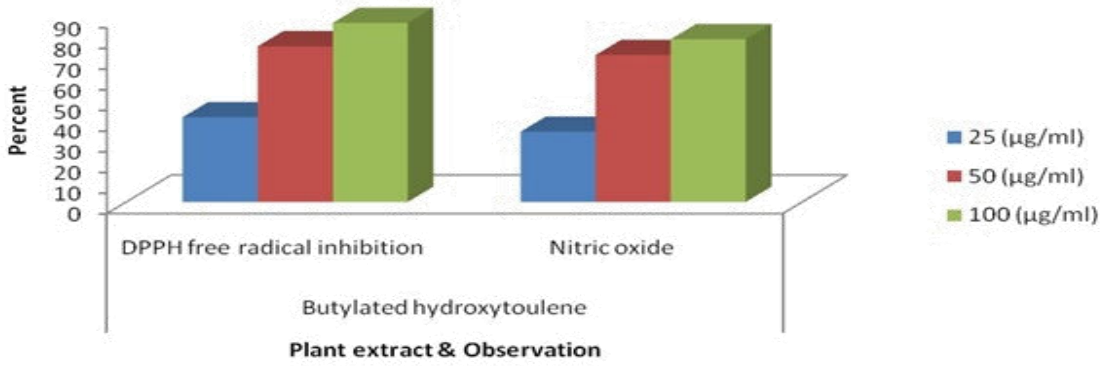

Fig. 3: *In-vivo* antioxidant activity animal study of standard solution of butylated hydroxytoluene (BHT) of plant extract

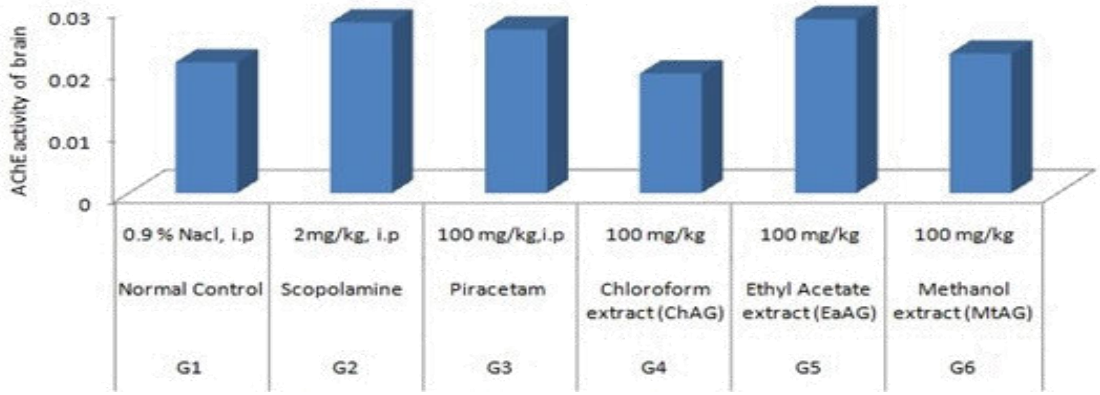

Fig. 4: Effect of plant extract for treatment on acetyl cholinesterase activity of brain (moles of substrate hydrolysed/min/gm tissue)

3.2.3. Acetyl cholinesterase activity

The result of scopolamine group showed very important raise in AchE activity with mean±SEM values 0.03717 ±0.0021. This result was compared to control group 0.02283±0.0024 for calculation of best result. The rats treated with extract demonstrated a moderate decrease in AChE activity with 0.02817±0.0021. It was compared to scopolamine group whereas a significant decrease in AChE activity is also observed in the groups treated with MEPP 300 and MEPP 500 mg/kg b.w. with 0.03383±0.0020. If the result compared to scopolamine group, value was 0.02617±0.0025 (table 7 and fig. 5). Values are expressed as Mean + SEM (n=6), by one way ANOVA followed by Newman keul multiple test [19-20].

Where, * represents significant at $p<0.05$, **represents highly significant at $p< 0.01$, and ***represents very significant at $p<0.001$.

a D-galactose induced ageing followed by Scopolamine induced amnesia group was significantly different from Normal control group.

b Treated group were significantly different from Dgalactose induced ageing followed by Scopolamine induced amnesia group.

3.2.4. Open-Field test

The plant Dill; *Anethum graveolens* Linn. fruit extract was evaluated on Mouse Locomotive Activities in the Open Field. As shown in table 8 and fig.6, no significant effect of same type of extract of *Anethum graveolens* Linn. (Fruit; 100 mg/kg) on mouse locomotive activities was observed in the open-field test. But galantamine 3 mg/kg reduced the total distance significantly compared with the aged control group ($P < 0.05$).

3.2.5. Morris water maze test

The plant Dill; *Anethum graveolens* Linn. fruit extract was evaluated on Mouse Locomotive Activities in the Aged Mice in Morris Water Maze Test. As shown in table 9 and fig. 7, no significant effect of same type of extract of *Anethum graveolens* Linn. (Fruit; 100 mg/kg) on mouse locomotive activities was observed, there were no significant differences on swimming speed in the whole training trials among all the groups.

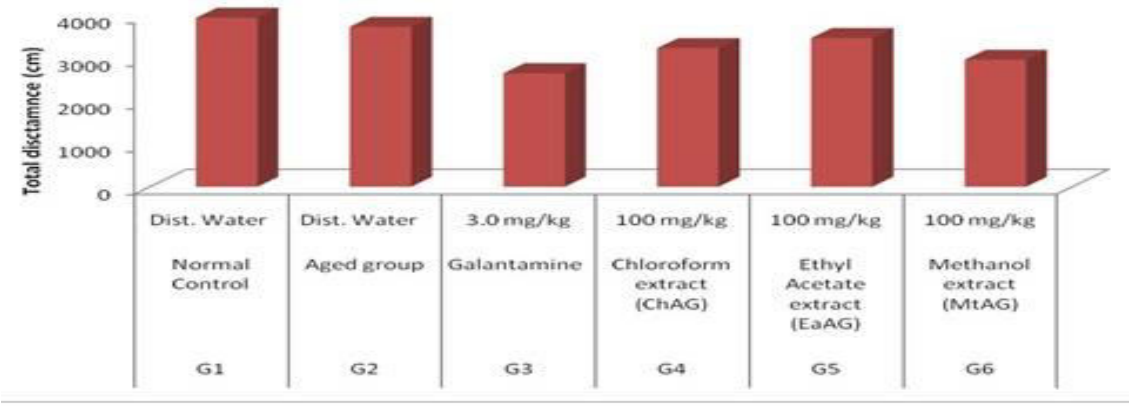

Fig. 5: Effect of plant extract on locomotive activities of mice, the total distance traveled by mice was measured after repeated administrations for 4 weeks.

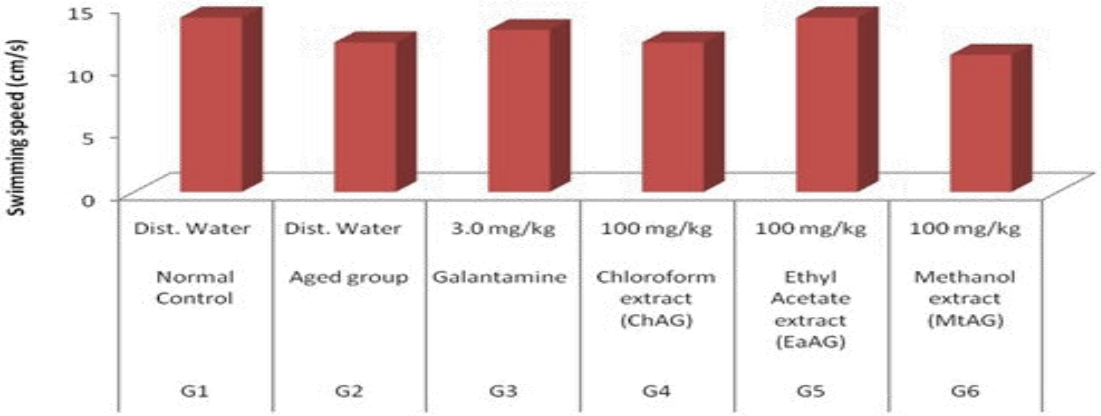

Fig. 6: Effect of plant extract on the acquisition phase in MWM tests of the aged mice, training trials were carried out on day 2 to day 7 of behavioral tests

Table 7: Effect of drug treatment on Acetyl cholinesterase activity of brain (moles of substrate hydrolysed/min/ gm tissue)

Group of animals	Treatment	Dose	AChE activity of brain
G1	Normal Control	0.9 % Nacl, i.p	0.0211343
G2	Scopolamine	2mg/kg, i.p	0.027564
G3	Piracetam	100 mg/kg,i.p	0.026435
G4	Chloroform extract (ChAG)	100 mg/kg	0.019345
G5	Ethyl Acetate extract (EaAG)	100 mg/kg	0.028123
G6	Methanol extract (MtAG)	100 mg/kg	0.0225423

*Values are expressed as Mean + SEM (n=6), by one way ANOVA followed by Newman keul multiple test. Where, * represents significant at p<0.05, ** represents highly represents very significant at p<0.001.significant at p< 0.01, and ****

Table 8: Effect of plant extract on locomotive activities of mice after repeated administrations for 4 weeks

Group of animals	Treatment	Dose	Total distance (cm)
G1	Normal Control	Dist. Water	3956
G2	Aged group	Dist. Water	3743
G3	Galantamine	3.0 mg/kg	2657
G4	Chloroform extract (ChAG)	100 mg/kg	3245
G5	Ethyl Acetate extract (EaAG)	100 mg/kg	3481
G6	Methanol extract (MtAG)	100 mg/kg	2974

3.2.6. Aged mice in step-down task

In the acquisition trials, the aged control mice revealed marked differences as compared with the normal control mice (P< 0.05 or P< 0.01). As shown Dill; Satahva; *Anethum graveolens* Linn. (Fruit) extract and galantamine (3 mg/kg) could shorten the latency and the time spent on the electric grid and increase the time spent in the safety zone significantly (P < 0.01 or P< 0.05). In the consolidation trials, the aged mice had longer time on the electric grid than the normal control mice (P < 0.01). However, no significant differences were observed in all groups on the time in safety zone. In the retrial tests, the aged control mice still displayed significant differences for the time spent in the safety zone, and the time spent on the electric grid as compared with the normal control mice. The treatments of plant extract *Anethum graveolens* Linn. (Fruit) and galantamine were performed and result of learning and memory showed in table 10 and fig. 8., ethyl acetate extract of plant extract in the dose of (100 mg/kg) have best result ($P < 0.01$ or $P < 0.05$).

Table 9: Effect of plant extract on the acquisition phase in MWM tests of the aged mice, training trials were carried out on day 2 to day 7 of behavioral tests

Group of animals	Treatment	Dose	Swimming speed (cm/s)
G1	Normal Control	Dist. Water	14
G2	Aged group	Dist. Water	12
G3	Galantamine	3.0 mg/kg	13
G4	Chloroform extract (ChAG)	100 mg/kg	12
G5	Ethyl Acetate extract (EaAG)	100 mg/kg	14
G6	Methanol extract (MtAG)	100 mg/kg	11

Table 10: Effect of plant extract on memory deficits of the aged mice in the step-down passive avoidance test

Group of animals	Treatment	Dose	Time on electric grid (s)
G1	Normal Control	Dist. Water	71
G2	Aged group	Dist. Water	16
G3	Galantamine	3.0 mg/kg	31
G4	Chloroform extract (ChAG)	100 mg/kg	41
G5	Ethyl Acetate extract (EaAG)	100 mg/kg	51
G6	Methanol extract (MtAG)	100 mg/kg	36

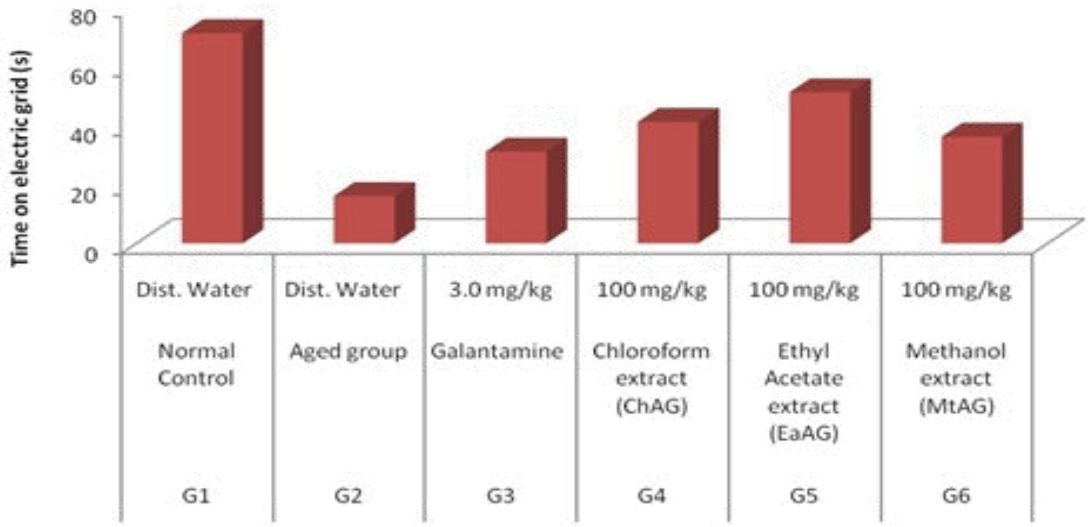

Fig. 7: Effect of plant extract on memory deficits of the aged mice in the step-down passive avoidance test

4. CONCLUSION

Memories have played justification criteria of personality of living things. It is identified by the behavior of person at every second by reminding own historical events and its outcomes. The cognitive dysfunction also known as brain fog, is the loss of intellectual functions such as thinking, remembering, and reasoning of sufficient severity to interfere with daily functioning. The plants Dill fruit have been found to possess number of therapeutic activities in literature. It also has valuable phytochemicals and this plant is suggested for present investigations. A systematic approach is necessary in pharmacognostic study, which helps in confirmation and determination of identity, purity and quality of a crude drug. *Anethum graveolens* Linn. fruits are in oval shape, dark brown colour, aromatic odour and having warm taste. Physicochemical parameter such as ash values *i.e.* total ash, acid insoluble ash and water soluble ash, extractive values, Loss on drying and pH of all selected plant drugs were performed. Ash values of crude drug provide an idea about the inorganic composition or earthy matter and other impurities present in drug. The extractive values are mainly useful for the determination of adulterated or exhausted drug. All parameters of selected drugs found within the limit as per API. Extracts (chloroform, ethyl acetate, and methanol extract) of selected plant drugs obtained by continuous soxhlet were subjected to qualitative phytochemical tests to identify the presence of secondary metabolite *i.e.* alkaloids, glycosides, tannins, flavonoids, sterols, fats, oils, phenols and saponins present. Preliminary phytochemical screening of plant *Anethum gravelosum* showed the presence of triterpenes in chloroform extract; triterpenes, tannins and flavanoids in ethyl acetate extract; carbohydrate, tannin flavanoid, protein and saponin in methanol extract. The experiments were performed on Swiss albino mice weight 20-25g or albino rats weight 110-150g. The animals were utilized separately for various experiments to be performed. The plant *Anethum graveolens* Linn. fruits extract were evaluated for memory enhancement potentials using the various in-vivo models. The plant *Anethum graveolens* Linn. fruits extract were evaluated for memory enhancement potentials using the various in-vivo models. The plant Dill; Satahva; *Anethum graveolens* Linn. (Fruit) ethyl acetate extract showed best memory enhancement property than other extract of same plant. The findings of *in-vivo* models indicate that treatment with ethyl acetate extract of plant did not cause significant change in the cortex of brain and have no side effect also. The result of the memory-enhancing effects were used for further study with these studies *i.e.* anti-inflammatory activity, antioxidant activity (Elevated plus Maze Model), acetylcholine Esterase inhibitory activity (Elevated plus Maze Model), open-field test, morris water maze test and step-down test on normal and aged mice. The tests showed the impaired spatial memory of the aged mice.

The proposed study were confirmed that ethyl acetate extract of plant have valuable active constituent, which took important play for anti-inflammatory and anti-oxidant activity in the brain tissue of the aged mice. The treatment of above discussed extract of plant on animals did not cause significant change in the cortex of brain and have no side effect and could be used for memory enhancement.

Conflict of interest

None declared

5. REFERENCES

1. Mayford M, Siegelbaum SA, Kandel E R. *Cold Spring Harbor Perspectives in Biology,* 2012; **4**(6):a005751-a005751.
2. Stern SA, Alberini CM, *Systems Biology and Medicine*, 2012; **5(1):**37-53.
3. Stern AS, Alberini CA, *WIREs Syst Biol Med*, 2013; **5:**37-53.
4. Claypoole KH, Noonan C, Mahurin RK, Goldberg J, Erickson T, Buchwald D, *Neuropsychology*, 2007; **21:**507-513.
5. Wallin MT, Wilken JA, Kane R, *J Rehabil Res Dev*, 2006; **43:** 63-72.
6. Glass JM, Park DC, *Curr Rheumatol Rep*, 2001; **3:**123-127.
7. Evans MA, Golomb BA, *Pharmacotherapy*, 2009; **29:**800-811.
8. Cooper C, Li R, Lyketsos C, Livingston G, *The British Journal of Psychiatry*, 2013; **203:**255-264.
9. Mesripour A, Rafieian-Kopaei M, Bahrami B, *Res Pharm Sci.*, 2016; **11:**145-151.
10. Jana Sand Shekhawat G, *Pharmacognosy Reviews*, 2010; **4:**179-184.
11. Oshaghi EA, Khodadadi I, Tavilani H, Goodarzi M, *Intern J of App Bas Med Res*, 2016; **6:**111-114.
12. Winter CA, Risley EA, *Proc. Soc. Exp. Bio. Med.,* 1962; **111:**545-549.
13. Turner RA, *Academic Press*, New York, 1965; 158.
14. Gopalkrisna B, Sutar PS, Akki KS, Gadad PC,

Hukkeri VI. *Indian Drugs*, 2006; **43:**255-261.
15. Raju TR, Kutty BM, Sathyaprabha TN, Shanakranarayana BS, *National Institute of Mental Health and Neuro Sciences*, 2004;142-144.
16. Guenther V, Klaus S, *Toxicol applied pharmacol,* 1970; **16:**764-772.
17. Dang H, Chen Y, Liu X, *Neuro-Psychopharmacology and Biological Psychiatry*, 2009; **33:**1417-1424.
18. Wang Q, Sun LH, Jia W, *Phytotherapy Research*, 2010; **24:**1748-1754.
19. Singh S, Dharamveer, Kulshreshtha M. *Cent Nerv Syst Agents Med Chem.,* 2019; **19:**125-132.
20. Pritchett D, Taylor AM, Barkus C, Engle SJ, Brandon NJ, Sharp T, Foster RG, Harrison PJ, Peirson SN, Bannerman DM. *Eur J Neurosci*. 2016; **43:**979-989.