

# Evaluation of Traditional Herb Extract *Salvia officinalis* in Treatment of Alzheimer's Disease

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## ABSTRACT

**Aim and Objective:** Alzheimer's disease is progressive neurodegenerative disorder which affects older individuals. It is the most common cause of dementia and it is associated with the presence of senile plaques which are deposition of beta- amyloid protein in the hippocampus area of the brain. Medicinal plants have played vital role in world health. In spite of the great advances observed in modern medicine in recent decades, plants still make an important contribution to health care. The present study is done to evaluate *Salvia officinalis*, for *in vivo* study on Alzheimer's disease induced mice. **Materials and Methodology:** Memory Enhancing Activity, Conditioned avoidance test, Y-maze spontaneous alternation test, Elevated Plus Maze, Morris Water Maze, Measurement of Locomotor Activity were done to assess memory & cognitive functioning. The isolated brain homogenate is estimated for reduced glutathione content, Acetyl cholinesterase Activity, Superoxide dismutase assay (SOD), Lipid peroxidation assay (TBARS), Glutathione peroxidase assay (GSH-Px) and Histopathology examination of brain was performed and analyzed. **Results and Discussion:** The elevated level of enzymes and decreased level of tissue antioxidant markers were observed in treatment comparative to piracetam treatment group. While 300 mg/kg extract significantly reduced the elevated levels of the enzymes and also significantly increased the tissue antioxidant levels, while decreased the glutathione levels when compared with the control. **Conclusion:** The histopathological study confirmed the recovery. The herbal extract (150 and 300 mg/kg) has shown effectiveness against Alzheimer's disease.

**Key words:** *Salvia officinalis*, Alzheimer's disease, Locomotor activity, Histopathological assessment.

## INTRODUCTION

Herbal medicinal products are defined as the study or use of medicinal herbs to prevent and treat diseases and ailments or to promote health and healing (or) a drug or preparation made from a plant or plants and used for any of such purposes. The use of natural products is in human diseases such as cardiovascular disease, cancer, and neurodegenerative disease is now a fruitful supporting therapy. Alzheimer's disease is progressive neurodegenerative disorder which affects older individuals. It is the most common cause of dementia. It is associated with the presence of senile plaques which are deposition of beta- amyloid protein in the hippocampus area of the brain. The contributing factors are not known yet, but several threads have been identified by the scientists who can link the factors like Age, Genetic history, Lifestyle, Heredity, Vascular risk factors, Head injury and Diet. The demonstration of damage to the cholinergic pathways in the brain leads to great interest in drug development. Acetylcholinesterase inhibitors are usually prescribed to treat AD. These drugs help in enhancing cognitive functions such as memory and thoughts. These medicines are effective in patients with mild to moderate AD. Tacrine (a cholinesterase inhibitor) at a high dose (160 mg/d) was reportedly used in the treatment of

AD. Tacrine was investigated in both clinical trials and neuropsychological test scores, in a 30 week randomized placebo controlled trial. Anti-oxidants are effective for AD because they aid in reducing the free radicals that damage the brain cells. Drugs involved with the CNS may have general stimulatory or depressant action with anticonvulsant and psychopharmacological activities. Memory deficit is a major global health problem. Current therapies are inadequate and have numerous adverse effects. There is an urgent need for possible alternative treatments for AD and memory deficit. Various medicinal plants are prescribed to enhance the memory. We have reviewed the literature on medicinal plants used in the treatment of AD and memory deficit.

Herbal medicine has long been used in China as therapy for dementia. *The Complete Work of Jingyue* published in 1624 contains the earliest known description in the world of a herbal therapeutic strategy for dementia. In the past 10 years, however, herbal drugs have seldom been approved for use alone in treating dementia. Overall, systematic review has identified a few single herbs and herbal formulations as possible effective medicine for AD. According to the current evidence, some of these therapies show promising results in terms of their cognitive benefits.

*S. officinalis* has numerous common names. Some of the best known are sage, common sage, garden sage,

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golden sage, kitchen sage, true sage, culinary sage, Dalmatian sage, and broadleaf sage. Cultivated forms include purple sage and red sage.

*S. officinalis* was described by Carl Linnaeus in 1753. It has been grown for centuries in the Old World for its food and healing properties, and was often described in old herbals for the many miraculous properties attributed to it. The specific epithet, *officinalis*, refers to the plant's medicinal use the *S. officinalis* was the traditional storeroom of a monastery where herbs and medicines were stored. *S. officinalis* has been classified under many other scientific names over the years, including six different names since 1940 alone. It is the type species for the genus *Salvia*.

The plant had a high reputation throughout the Middle Ages, with many sayings referring to its healing properties and value. It was sometimes called *S. salvatrix* (sage the savior) and was one of the ingredients of Four Thieves Vinegar, a blend of herbs which was supposed to ward off the plague. Dioscorides, Pliny, and Galen all recommended sage as a diuretic, hemostatic, emmenagogue, and tonic. John Gerard's Herball (1597) states that sage "is singularly good for the head and brain, it quicken the senses and memory, strengthen the sinews, restore health to those that have the palsy, and take away shake trembling of the members." In past centuries it was also used for hair care, insect bites and wasp stings, nervous conditions, mental conditions, oral preparations for inflammation of the mouth, tongue and throat, and also to reduce fevers.

According to Peter Rogers' team at Bristol University, researchers have concluded that extracts of sage can enhance cognitive performance. This was compared to the similar effect of the caffeine found in tea and coffee. Due to some of its chemical compound properties that allow it to deal with free radicals in a person's digestive system, it can be used in treating various digestive disorders such as flatulence, poor digestion, and bloating.

There was a study done in 2001 to test *S. officinalis*' antioxidant activities, and the results showed that rosmarinic acid, along with other chemical compounds, showed affective antioxidant activity. Antioxidant activity is important because many serious diseases such as brain dysfunction, cancer, heart diseases, and immune system decline have the possibility of resulting from cellular impairment caused by free radicals. Having high amounts of antioxidants in a person's diet may aid in disease prevention by getting rid of the free radicals.

## MATERIALS

### Experimental animals

Swiss albino mice (20-25 g) obtained and were maintained under standard condition in animal house. The animals were maintained under standard conditions of temperature ( $23 \pm 2^\circ\text{C}$ ), humidity (35-60%), and 12:12 light and dark ratio. The animals were placed in polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water *ad libitum*.<sup>1-15</sup> IAEC No: 1688/PO/E/2013/CPCSEA.

### Plant material

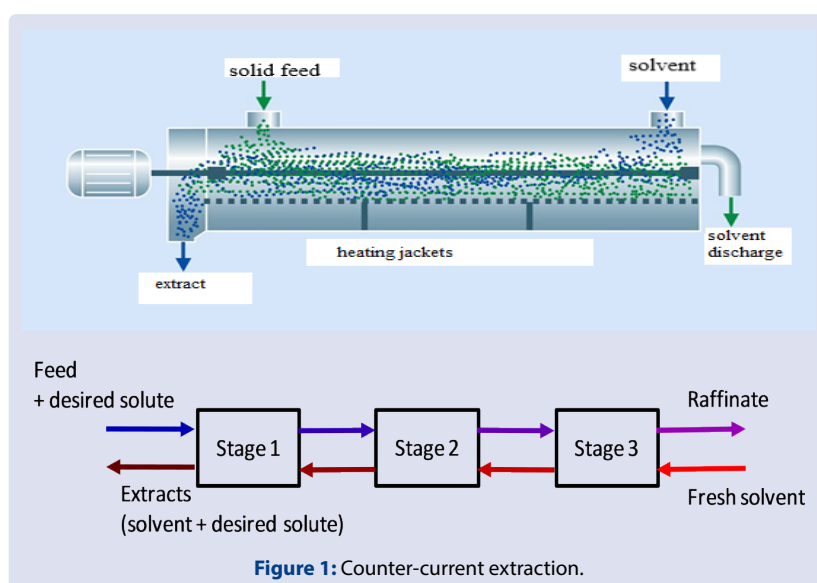
The fresh parts of *Salvia officinalis*, was collected from local areas of Tirupathi, Andrapradesh, India and authenticated by Prof. Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupathi. The plant was dried in shade and was ground to get fine powder.

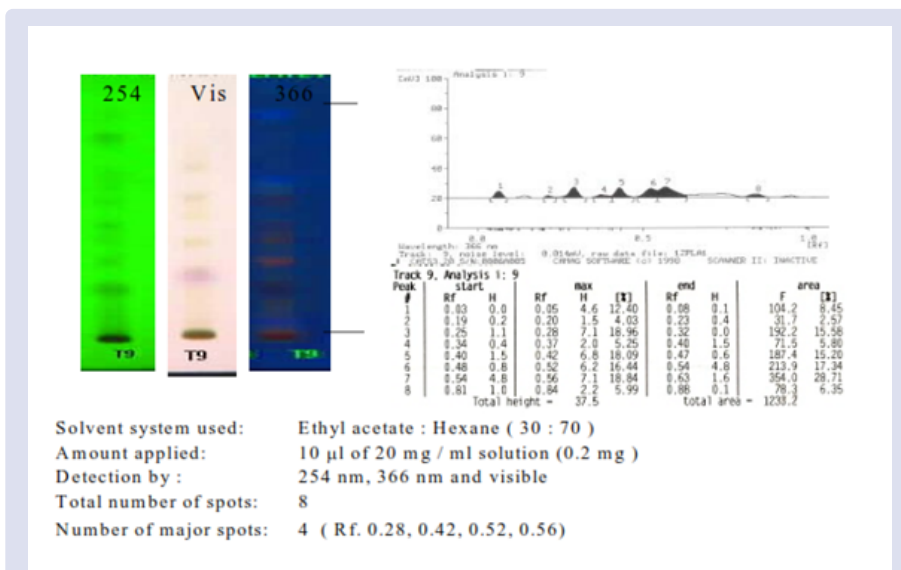
## METHODS

### Extraction of plant material by means of counter current extraction procedure<sup>16</sup>

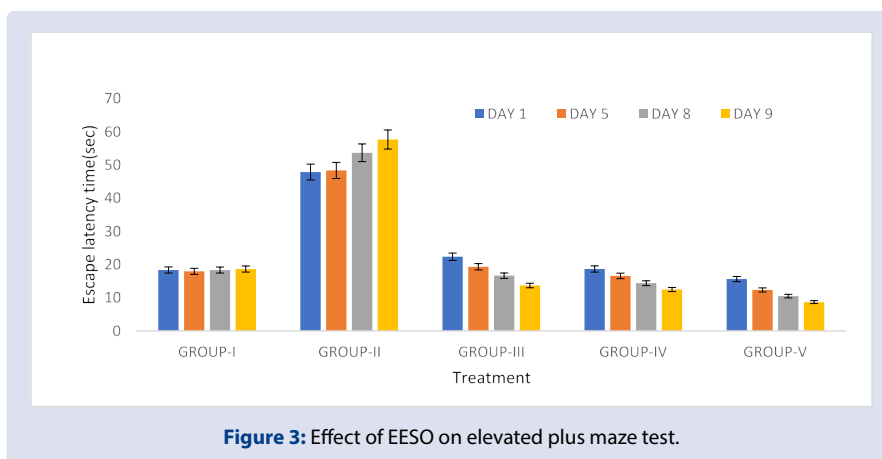
This extraction process has significant advantages<sup>16</sup>:

- i) A unit quantity of the plant material can be extracted with much smaller volume of solvent as compared to other methods like maceration, decoction, and percolation.
- ii) CCE is commonly done at room temperature, which spares the thermo labile constituents from exposure to heat which is employed in most other techniques.
- iii) As the pulverization of the drug is done under wet conditions, the heat generated during comminution is neutralized by water. This again spares the thermo labile constituents from exposure to heat.
- iv) The extraction procedure has been rated to be more efficient and effective than continuous hot extraction.
- v) Prepared extracts using above methods will be vacuum dried to remove any solvent remaining.

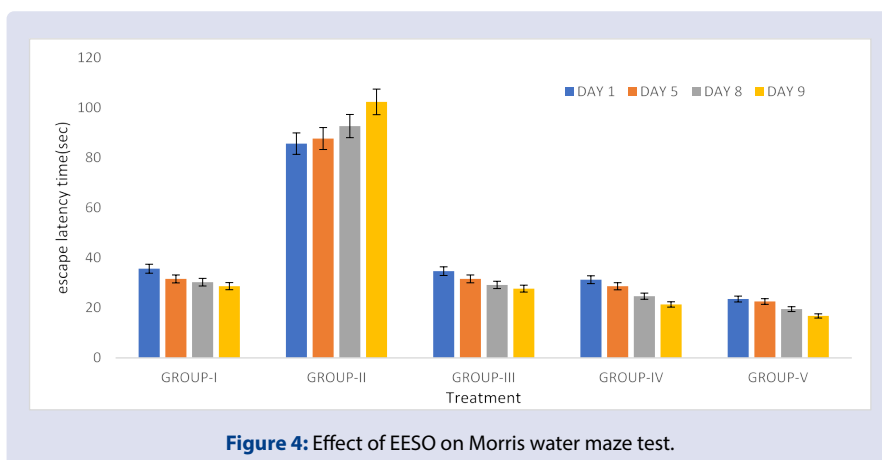




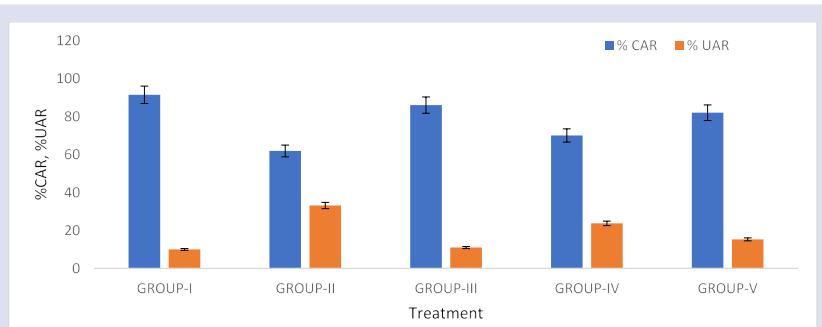
**Figure 2:** TLC of ethanol extract of *salvia officinalis* detection at visible, 254 and 366 nm and its HPTLC densitogram detection at 366 nm.



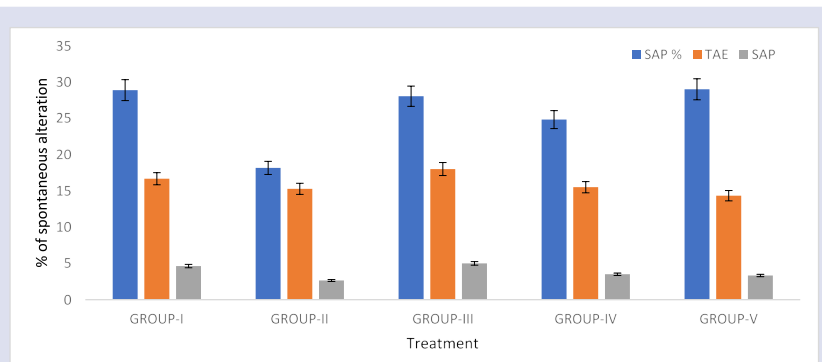
**Figure 3:** Effect of EESO on elevated plus maze test.



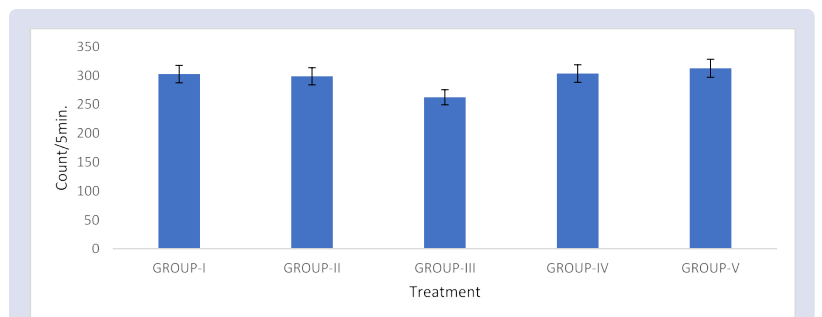
**Figure 4:** Effect of EESO on Morris water maze test.



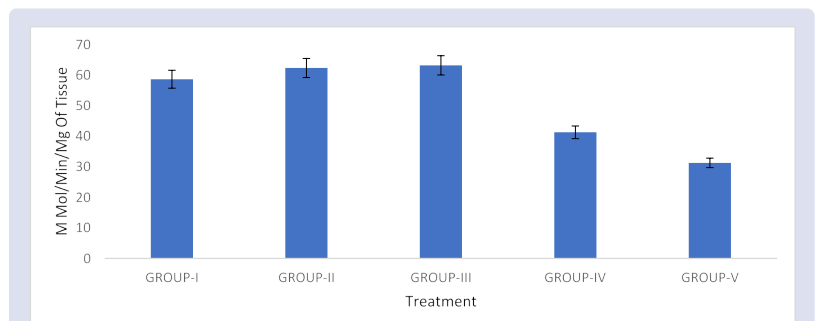
**Figure 5:** Effect of EESO on the active avoidance learning in scopolamine-induced demented mice.



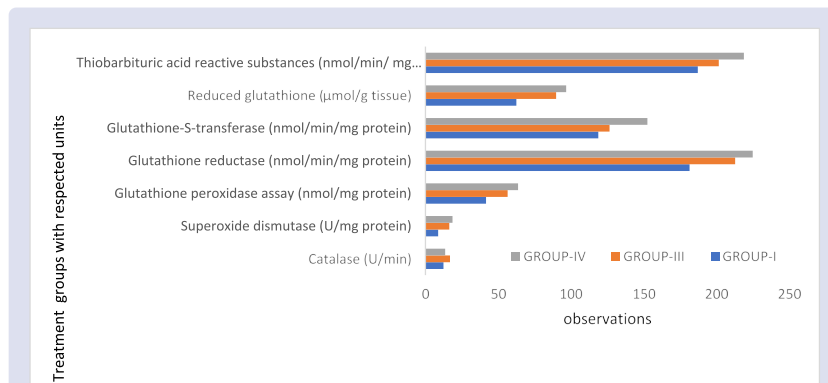
**Figure 6:** Effect of EESO on Y-maze behavioral test in scopolamine-induced demented mice.



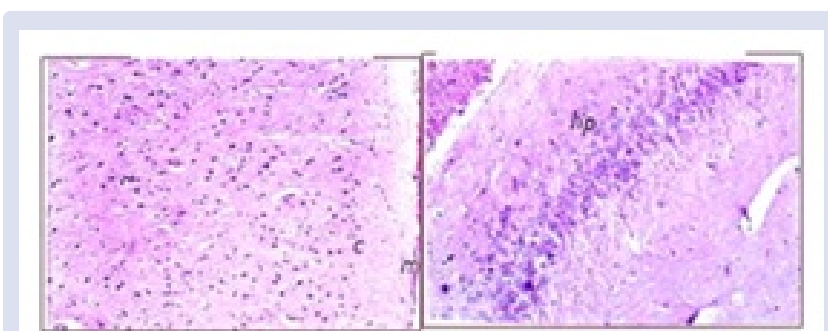
**Figure 7:** Measurement of locomotor activity.



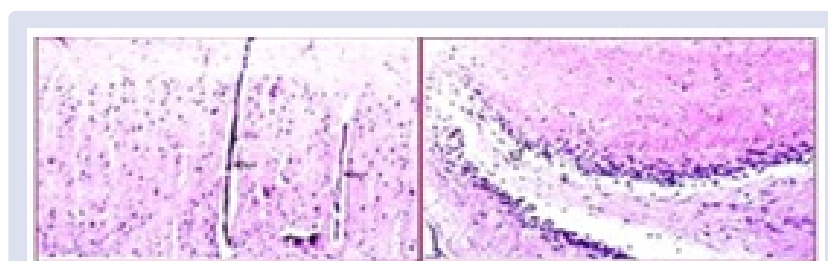
**Figure 8:** Brain acetylcholinesterase activity.



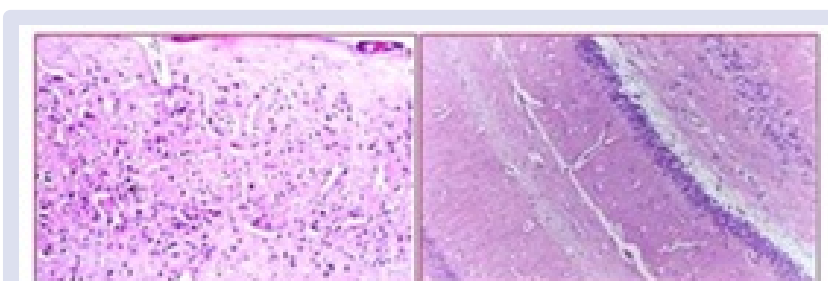
**Figure 9:** Effect of EESO administration for 7 days in biochemical parameters of animal brain antioxidant status.



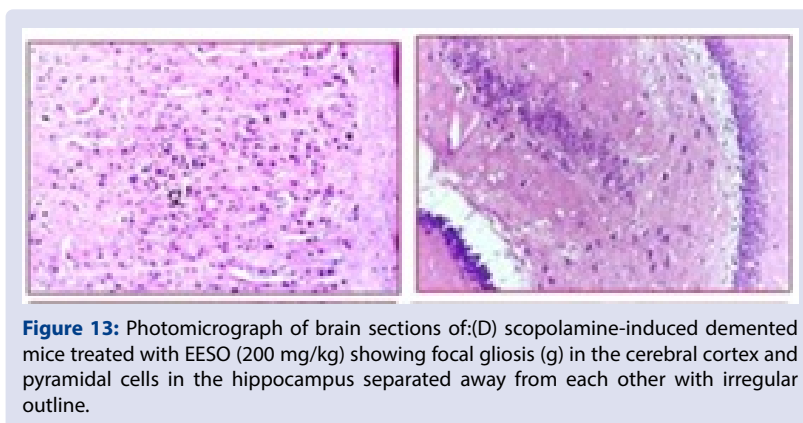
**Figure 10:** Photomicrograph of brain sections of: (A) control mice showing the normal histological structure of the meninges (m), cerebral cortex (c) and hippocampus (hp).



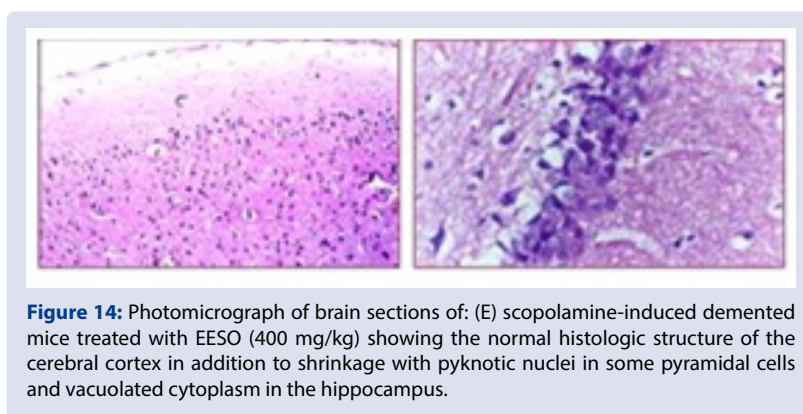
**Figure 11:** Photomicrograph of brain sections of: (B) scopolamine induced demented mice showing severe congestion in the blood capillaries with perivascular edema (arrow) in the cerebral cortex together with edema and encephalomalacia in the hippocampus (d).



**Figure 12:** Photomicrograph of brain sections of: (C) Scopolamine induced demented mice treated with piracetam showing diffuse gliosis in the cerebral cortex and shrinkage with pyknotic nuclei in some pyramidal cells in the hippocampus.



**Figure 13:** Photomicrograph of brain sections of:(D) scopolamine-induced demented mice treated with EESO (200 mg/kg) showing focal gliosis (g) in the cerebral cortex and pyramidal cells in the hippocampus separated away from each other with irregular outline.



**Figure 14:** Photomicrograph of brain sections of: (E) scopolamine-induced demented mice treated with EESO (400 mg/kg) showing the normal histologic structure of the cerebral cortex in addition to shrinkage with pyknotic nuclei in some pyramidal cells and vacuolated cytoplasm in the hippocampus.

## Procedure

- 500gms of plant powder to be extracted is taken into the feed chamber.
- Then 2000ml of solvent is taken in to the solvent feed chamber.
- When the motor is turned on for mixing the solvent gets mixed with the plant powder.
- The solvent continuously flows through the plant powder chamber and gets collected at the opposite end of the solvent feed.
- The collected solvent is again introduced into the solvent feed chamber after filtering for the extract.
- This process is repeated for 12hours.
- Finally when the powder in the chamber gets reduced to 50% the process is stopped.
- And again repeated the same process with the collected extract with fresh solvent of 1000ml. and the filtrate from this second step is taken for drying using rota evaporator.

## Acute toxicity study

Acute toxicity study was carried using mice by up and down/staircase method as per OECD guidelines. The aim of this experiment was to determine the LD50 of the crude extract. Mice were randomly divided into five groups of 2 animals each. Group-1 served as control and received normal saline (10 ml/kg) while other groups (Group 2, 3, 4 and 5) were given different doses of crude extract in an ascending order that is, 100, 500, 1000, 1500 2000 mg/kg respectively. The mortality rate observed for 24 h. Since no mortality occurred, another five groups of mice were taken. All the doses administered by intra-peritoneal route. The highest dose that did not kill any animals and the lowest dose

that resulted in mortality were noted. LD50 was calculated from the geometric mean of these two doses.

## Disease induction and experimental design

Animals were classified into five groups (6 mice each). Treatments were given p.o. for 27 successive days. After the last dose of test agents on 17<sup>th</sup> day, all animals were i.p. injected with scopolamine hydro bromide (20 mg/kg) except the first group (control group). Animals were treated according to the selected doses of extracts in respected groups.<sup>17</sup>

## Study design for activity

Experimental animals: Swiss albino Mice (20-25g)

Animals were grouped randomly into 5 groups, five Mice each.

**Group1:** Vehicle control group - Mice treated with distilled water.

**Group 2:** control group - Mice were treated with scopolamine hydrobromide (20 mg/kg) i.p. injection on the initial day of experiment.

**Group 3:** Mice were treated with scopolamine hydrobromide (20 mg/kg) i.p., on the initial day of experiment along with the administration of Low dose of EESO200 mg/kg

**Group 4:** Mice were treated with scopolamine hydrobromide (20 mg/kg) i.p., on the initial day of experiment along with the administration of high dose of EESO400 mg/kg

**Group 5:** Standard control group -Mice were treated with scopolamine hydrobromide (20 mg/kg) i.p., along with the administration of Piracetam for 10 days.

The animals were administered with doses for seven days and parameters were evaluated.<sup>18</sup>

**Table 1: Preliminary phytochemical screening.**

Phytochemical	Salvia.officinalis
Steroid	-
Alkaloid	-
Tannin	+
Carbohydrate	+
Phenol	+
Flavonoids	+
Saponin	-
Reducing sugar	+
Protein	+
Amino acid(free)	-
Terpenoids	+
Coumarin	+

(+) Present. (-) Absent

**Table 2: Quantitative analysis of phytochemical constituents.**

Type of extract	Total phenolics (mg GAE/100 g DW)	Total flavonoids (mg GAE/100 g DW)	Total saponins (mg GAE/100 g DW)	Total alkaloids (mg GAE/100 g DW)	Essential oils (µg/ml)
EESO	31.15	18.46	-	-	62.65
MESO	21.34	12.54	-	-	53.45
HASO	22.51	12.34	-	-	55.62

(Milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g dw))

Note: depending upon the quantity of the chemical constituents present, only ethanolic extracts are preferred for the further studies.

**Table 3: Determination of percentage Yield of major compounds using HPTLC densitogram.**

S. No.	Extracts	Yield (% w/v)	Total number of spots	Number of major spots	Retention factor (Rf)	% Area (with reference to crude extract)	% Yield* of major compound
1.	MESO	13.0	8	3	0.28	16.22	0.51
					0.42	32.40	1.04
					0.52	37.73	1.21

**Table 4: Effect of EESO on elevated plus maze test.**

TREATMENT	Escape Latency Time (In Second) (Mean ± SEM)			
	DAY 1	DAY 5	DAY 8	DAY 9
GROUP-I	18.35 ± 1.25	17.95 ± 1.02	18.34 ± 1.05	18.64 ± 1.02
GROUP-II	47.86 ± 1.35	48.35 ± 1.06	53.67 ± 1.85	57.68 ± 1.09
GROUP-III	22.34 ± 1.52	19.32 ± 1.28	16.64 ± 1.04	13.67 ± 1.08
GROUP-IV	18.65 ± 1.34	16.58 ± 1.35	14.37 ± 1.05	12.48 ± 1.24
GROUP-V	15.62 ± 1.52	12.35 ± 1.31	10.48 ± 1.04	8.67 ± 1.04

N=6, Mean ± SEM.

**Table 5: Effect of EESO on Morris water maze test.**

TREATMENT	Escape Latency Time (In Second) (Mean ± SEM)			
	DAY 1	DAY 5	DAY 8	DAY 9
GROUP-I	35.65 ± 1.39	31.56 ± 1.25	30.27 ± 1.35	28.67 ± 1.56
GROUP-II	85.64 ± 1.54	87.68 ± 1.24	92.67 ± 1.48	102.34 ± 1.34
GROUP-III	34.67 ± 1.24	31.58 ± 1.65	29.16 ± 1.26	27.67 ± 1.34
GROUP-IV	31.24 ± 1.06	28.64 ± 1.24	24.67 ± 1.24	21.37 ± 1.38
GROUP-V	23.54 ± 1.08	22.54 ± 1.58	19.49 ± 1.57	16.78 ± 1.81

N=6, Mean ± SEM.

**Table 6: Effect of EESO on the active avoidance learning in scopolamine-induced demented mice.**

TREATMENT	Parameters	
	% CAR	% UAR
GROUP-I	91.43 ± 2.10	10.00 ± 1.83
GROUP-II	61.88 ± 3.65	33.13 ± 2.49
GROUP-III	70.00 ± 3.54	11.00 ± 2.45
GROUP-IV	86.00 ± 3.67	23.75 ± 4.73
GROUP-V	82.00 ± 4.64	15.30 ± 3.28

**Table 7: Effect of EESO on Y-maze behavioral test in scopolamine-induced demented mice.**

TREATMENT	Parameters		
	SAP %	TAE	SAP
GROUP-I	28.88 ± 0.66	16.67 ± 2.30	4.63 ± 0.68
GROUP-II	18.16 ± 1.45	15.27 ± 1.66	2.64 ± 0.51
GROUP-III	24.82 ± 1.61	15.50 ± 5.25	3.50 ± 1.61
GROUP-IV	28.04 ± 1.78	18.00 ± 4.97	4.00 ± 0.71
GROUP-V	29.00 ± 1.56	14.33 ± 1.54	3.33 ± 0.42

N=6, Mean ± SEM.

**Table 8: Effect of EESO on locomotor activity.**

S.NO	TREATMENT	Locomotor activity counts/5 min
1	GROUP-I	302.5 ± 1.26
2	GROUP-II	238.65 ± 1.34
3	GROUP-III	262.34 ± 1.29
4	GROUP-IV	303.48 ± 1.48
5	GROUP-V	312.54 ± 1.43

N=6, Mean ± SEM.

**Table 9: Effect of EESO on Brain acetylcholinesterase.**

S.NO	TREATMENT	Acetylcholinesterase Activity (M Mol/Min/Mg of Tissue)
1	GROUP-I	58.67 ± 1.95
2	GROUP-II	62.37 ± 1.27
3	GROUP-III	63.22 ± 1.85
4	GROUP-IV	41.31 ± 1.58
5	GROUP-V	31.29 ± 1.25

N=6, Mean ± SEM.

**Table 10: Effect of EESO administration for 7 days in biochemical parameters of animal brain antioxidant status.**

Treatment	Catalase (U/min)	Superoxide dismutase (U/mg protein)	Glutathione peroxidase assay (nmol/mg protein)	Glutathione reductase (nmol/min/mg protein)	Glutathione-S-transferase (nmol/min/mg protein)	Reduced glutathione (µmol/g tissue)	Thiobarbituric acid reactive substances (nmol/min/mg protein)
GROUP-I	12.35 ± 1.42	8.67 ± 1.75	41.59 ± 1.27	181.34 ± 1.6	118.67 ± 1.07	62.37 ± 1.08	186.95 ± 1.05
GROUP-III	16.84 ± 1.04	16.37 ± 1.48	56.37 ± 1.58	212.64 ± 2.0	126.37 ± 1.07	89.67 ± 1.05	201.37 ± 1.5
GROUP-IV	13.54 ± 1.06	18.54 ± 1.54	63.57 ± 1.4	224.68 ± 2.1	152.37 ± 1.08	96.58 ± 1.06	218.63 ± 1.2

N=6, Mean ± SEM.

## Models for memory enhancement in rats

### Conditioned avoidance test

The test was carried out using a shuttle box (Reflex-16, Columbus Instruments, USA) as described by Wadenberg ML *et al.* The shuttle box consisted of two grid floor compartments (29 × 29 × 26 cm) separated by a non-transparent partition with a single opening (8 × 4.5 cm). Light (12 W) attached to the ceiling of the shuttle box was used as conditioned stimuli; whereas, the unconditioned stimulus was a foot shock (0.8 mA) delivered through the metallic grid floor. mice were trained for three days prior to conduction of the experiment by subjecting them to the conditioned stimulus (light) followed by the unconditioned one (electric shock). After injection of scopolamine, each mice was placed in the shuttle box and allowed to adapt for 3 min. Following adaptation, the conditioned stimulus was presented for 10s prior to the unconditioned stimulus. If the mice crossed to the opposite compartment during 10 s of conditioned stimulus, the electric shock was avoided; otherwise failure of avoidance was recorded<sup>18</sup>.

### Y-maze spontaneous alternation test

The Y-maze test was performed as described by Wall and Messier. The maze was made of 3 identical arms, 40 cm long, 35 cm high and 12 cm wide, positioned at equal angles and labeled A, B, and C. mice were

placed at the end of one arm and allowed to move freely through the maze during a 5 min session. Spontaneous alternation was examined by visually recording the pattern of entrance into each arm in the maze for each mice. Arm entry was considered to be complete when the hind paws of the mice were completely placed in the arm. Alternation was defined as successive entries into the three arms on overlapping triplet set (i.e., ABC, BCA.....). Accordingly, the spontaneous alternation performance (SAP) score, spontaneous alternation percentage (SAP %) and total arm entries (TAE) were calculated.<sup>19</sup>

### Elevated plus maze

The procedure, technique, and end point for testing learning and memory were followed as per the parameters described earlier. The elevated plus maze for mice consisted of two open arms (16 cm × 5 cm) and two covered arms (16 cm × 5 cm × 15 cm) extended from a central platform (5 cm × 5 cm) and the maze was elevated to a height of 25 cm from the floor. On the first day, each mouse was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was defined as the time taken by the animal to move from the open arm into one of the covered arms with all its four legs. TL was recorded on the first day (i.e., 10th day of drug administration) for each animal. If the animal did not enter into one of the covered arm within 90sec, it was gently pushed into one of the two covered arms and TL

was assigned as 90 sec. The mouse was allowed to explore the maze for another 2 minutes and then returned to its home cage. Retention of this learned-task (memory) was examined 24 h (11th day) after the first day trial.<sup>20</sup>

### Morris water maze

The procedure, technique, and end point for testing memory were followed as per the parameters described earlier. Briefly, Morris water maze-(MWM) for mice consisted of a circular pool (60 cm in diameter, 25 cm in height) filled to a depth of 20 cm with water maintained at 25°C. The water was made opaque with nontoxic white colored dye. The tank was 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 submerged platform (with top surface 6 cm × 6 cm and painted in white) was placed inside the target quadrants (Q4 in present study) of this pool 1 cm below surface of water. The position of platform was kept unaltered throughout the training session. Each animal was subjected to four consecutive trials each day with a gap of 5 min for four consecutive days (starting from 6th day of drug administration to 9th day), during which they were allowed to escape on to the hidden platform and to remain there for 20 s. During the training session, the mouse was gently placed in the water between quadrants, facing the wall of pool with drop location changing for each trial, and allowed 120 sec to locate submerged platform. If the mouse failed to find the platform within 120 s, it was guided gently on to the platform and allowed to remain there for 20 s. Escape latency (EL) is the time taken by the animal to move from the starting quadrant to find the hidden platform in the target quadrant. EL was recorded on the 6th day to 9th day for each animal. Each animal was subjected to training trials for four consecutive days, the starting position was changed with each exposure as mentioned below and target quadrant (Q4 in the present study) remained constant throughout the training period.

- Day1 Q1 Q2 Q3 Q4.
- Day2 Q2 Q3 Q4 Q1.
- Day3 Q3 Q4 Q1 Q2.
- Day4 Q4 Q1 Q2 Q3.

On the fifth day (i.e., 10th day of drug administration), the platform was removed and mouse was placed in any of the three quadrants and allowed to explore the target quadrant for 300 s. Mean time spent in all the three quadrants that is, Q1, Q2, and Q3 was recorded. The mean time spent in the target quadrant in search of the missing platform was noted as index of retrieval or memory. The observer always stood at the same position. Care was taken not to disturb the relative location of water maze with respect to other objects in the laboratory.<sup>20</sup>

### Measurement of locomotor activity

To rule out the effects of the drugs on motor activity, horizontal locomotor activities of control and test animals were recorded for a period of 5 min using Medicraft Photoactometer, Model number 600-4D (INCO, Ambala, India). The photoactometer consisted of a square arena (30 × 30 × 25 cm) with wire mesh bottom, in which the animal moves. Six lights and six photocells placed in the outer periphery of the bottom in such a way that a single mouse can block only one beam. Technically its principle is that a photocell is activated when animals crossing the beam of light cut off the rays of light falling on the photocells. As the photocell is activated, a count is recorded. The photocells are connected to an electronic automatic counting device which counts the number of "cut offs."<sup>20</sup>

### Estimation of brain reduced glutathione content

The method for the assessment of GSH (mg/g wet tissue) in the brain homogenates was based on that of Beutler *et al.* Homogenates were de-proteinized with 5-sulfosalicylic acid (10%) for 30 min at 4 °C then centrifuged at 3000rpm for 15 min at 4°C. An aliquot of the acid soluble supernatant was diluted with phosphate buffer (0.3 M, pH = 7.7) and 5,5'-dithiobis-2-nitrobenzoic acid (1 mM) was added to the samples. The optical density of the produced colored product was determined at 412 nm.<sup>21</sup>

### Biochemical estimation

#### Collection of brain sample

Immediately after behavioural testing (retrieval) on elevated plus maze, animals sacrificed by cervical dislocation under light anaesthesia with diethylether. The whole brain carefully removed from the skull. For preparation of brain homogenate, the fresh whole brain 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 3000 rpm for 10 min at 4°C and the resultant cloudy supernatant liquid used for the estimation of brain acetylcholinesterase activity.<sup>21</sup>

#### Brain Acetylcholinesterase Activity<sup>22</sup>

Brain acetylcholinesterase estimated using the method of D.H. den Blaauwen *et al.* Briefly, 0.4 mL of brain homogenate was added to a test tube containing 2.6 mL of phosphate buffer. 0.1 mL DTNB reagent added to the above mixture and absorbance was noted at 412 nm. 0.02 mL of acetylcholine iodide solution added and again absorbance was noted 15 min thereafter. Change in absorbance per min was calculated.

The rate of hydrolysis of substrate was calculated using following formula:

- $R = \text{change in absorbance/min} \times 5.74 \times 10^{-4} / C_0$ ,
- $R = \text{rate of hydrolysis of acetylcholine iodide/min/mg tissue}$ ,
- $C_0 = \text{weight of tissue homogenate in mg/mL}$ .

#### Superoxide dismutase assay (SOD)<sup>22</sup>

SOD activity was estimated by the method of D.H. den Blaauwen *et al.* Reaction mixture of this method contained: 0.1 ml of phenazine methosulphate (186 μmol), 1.2 ml of sodium pyrophosphate buffer (0.052 mmol; pH 7.0), 0.3 ml of the supernatant after centrifugation (1500 × g for 10 min followed by 10,000 × g for 15 min) of homogenate was added to the reaction mixture. Enzyme reaction was initiated by adding 0.2 ml of NADH (780 μmol) and stopped after 1 min by adding 1 ml of glacial acetic acid. Amount of chromogen formed measured by recording color intensity at 560 nm. Results are expressed in units/mg protein.

#### Estimation of a lipid peroxidation assay (TBARS)<sup>23</sup>

The assay for lipid peroxidation was carried out following the modified method of M. Mihara *et al.*. The reaction mixture in a total volume of 1.0 ml contained 0.58 ml phosphate buffer (0.1 mol; pH 7.4), 0.2 ml homogenate sample, 0.2 ml ascorbic acid (100 mmol), and 0.02 ml ferric chloride (100 mmol). The reaction mixture was incubated at 37°C in a shaking water bath for 1 h. The reaction stopped by addition of 1.0 ml 10% trichloroacetic acid. Following addition of 1.0 ml 0.67% thiobarbituric acid, all the tubes were placed in boiling-water bath for 20 min and then shifted to crushed ice-bath before centrifuging at 2500 × g for 10 min. The amount of TBARS formed in each of the

samples assessed by measuring optical density of the supernatant at 535 nm using a spectrophotometer against a reagent blank. The results expressed as nmol TBARS/min/mg tissue at 37°C using a molar extinction coefficient of  $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ .

### Glutathione peroxidase assay (GSH-Px)<sup>24</sup>

Glutathione peroxidase activity assayed by the method of Mohandas *et al.* The reaction mixture consisted of 1.49 ml phosphate buffer (0.1 mol; pH 7.4), 0.1 ml EDTA (1 mmol), 0.1 ml sodium azide (1 mmol), 0.05 ml glutathione reductase (1 IU/ml), 0.05 ml GSH (1 mmol), 0.1 ml NADPH (0.2 mmol), 0.01 ml H<sub>2</sub>O<sub>2</sub> (0.25 mmol) and 0.1 ml of homogenate in a total volume of 2 ml. The disappearance of NADPH at 340 nm recorded at 25°C. Enzyme activity calculated as nmol NADPH oxidized/min/mg protein using a molar extinction coefficient of  $6.22 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ .

### Histopathologic examination of brain tissues

Histopathologic assessment performed on the brains of 2–3 mice randomly selected from each group. Brains were immediately fixed in 10% phosphate buffered formaldehyde, embedded in paraffin, and 5 µm longitudinal sections were performed. The sections were stained with hematoxylin and eosin (H&E) and examined microscopically (250-350x).<sup>25</sup>

### Statistical analysis

Results expressed as Mean ± S.D. All the results compared with control subject tone-way analysis of variance (ANOVA), followed by the dunnet test using Graph Pad Prism Software 6 version.

P Values <0.05 were as considered statistically significant.

## RESULTS

### % yield value of ethanol extract from Aerial Parts of *Salvia officinalis* plant

Percentage yield of ethanol extract found to be 20%.

### Preliminary photochemical screening

Investigation revealed the presence of Tannin, Carbohydrate, Phenol, Flavonoid, Reducing sugar, protein, Terpenoids and Coumarin.

### TLC studies

The selected extracts subjected to TLC studies and the results were as shown below.

## ISOLATION AND CHARACTERIZATION OF COMPOUNDS

Ethanol extract of *Salvia officinalis* further subjected to column fractionation using normal phase silica gel column. Those column fractions further purified by Preparative TLC, using Silica gel-G as a stationary phase. From the ethanol extract two major compounds were isolated and identified as rosmarinic acid and carnosic acid. The identification was done using marker compound and NMR spectrum. Another third compound is carnosol.

## REPORT FOR ACCUTE TOXICITY STUDIES

### LD50 values of different fractions of *Salvia officinalis* of E-Ethanol extract

2000mg/kg is considered as the lethal dose of the test compound as the animals showed severe side effects. The required amount from

the 2000mg/kg will be calculated (200mg/kg & 400mg/kg) and will be administered to animals.

### Effect of EESO on elevated plus maze test

#### Treatment schedule

Animals were divided in 4 groups; in each group 6 animals were used. Subgroup 01 (vehicle control: Acacia suspension 1 ml/kg oral), group 02 (Scopolamine (20 mg/kg)), group 3 (EESO 200 mg/kg, oral), group 4 (EESO 400 mg/kg, oral), group 5 standard (piracetam 200 mg/kg intraperitoneal).

#### Inference

The treatment showed positive reports (memory response) as the mice of group-IV showed significant response times ( $12.48 \pm 1.24$ ) compared to the standard drug ( $8.67 \pm 1.04$ ) still the results of standard are better but as for a herbal extract the obtained timings are good for consideration and improvement and the group-III animals also showed good results. These results show that the extracts are improving the memory functions of the animals.

### Effect of EESO on Morris water maze test

#### Treatment schedule

Animals were divided in 4 groups; in each group 6 animals were used. Subgroup 01 (vehicle control: Acacia suspension 1 ml/kg oral), group 02 (Scopolamine (20 mg/kg)), group 3 (EESO 200 mg/kg, oral), group 4 (EESO 400 mg/kg, oral), group 5 standard (piracetam 200 mg/kg intraperitoneal).

#### Inference

In this test the animals kept in a water maze with four parts separated by a small thread where one part has a base for standing other parts are filled with water mice are placed in the water maze and they must find and the part with the base and stand on it for 20s after the training exercises the treatment is given. As shown in the above table Scopolamine treated mice (group-I) were taken as disease control and the obtained results are compared to it the group-IV mice showed  $21.37 \pm 1.38$  sec of escape time by the 9<sup>th</sup> day. And the animals of group-III showed  $27.67 \pm 1.34$  sec of escape time by the 9<sup>th</sup> day. These results show that the extracts are improving the memory functions of the animals.

#### Conditioned avoidance test

The test was carried out using a shuttle box (Reflex-16, Columbus Instruments, USA) as described by Hinrichs *et al.* The shuttle box consisted of two grid floor compartments (29 × 29 × 26 cm) separated by a non-transparent partition with a single opening (8 × 4.5 cm). Light (12 W) attached to the ceiling of the shuttle box was used as conditioned stimuli; whereas, the unconditioned stimulus was a foot shock (0.8 mA) delivered through the metallic grid floor. Mice were trained for three days prior to conduction of the experiment by subjecting them to the conditioned stimulus (light) followed by the unconditioned one (electric shock). After injection of scopolamine, each mice was placed in the shuttle box and allowed to adapt for 3 min. Following adaptation, the conditioned stimulus presented for 10 s prior to the unconditioned stimulus. If the mice crossed to the opposite compartment during 10 s of conditioned stimulus, the electric shock was avoided; otherwise failure of avoidance was recorded.

#### Treatment schedule

Animals were divided in 4 groups; in each group 6 animals were used. Subgroup 01 (vehicle control: Acacia suspension 1 ml/kg oral), group 02 (Scopolamine (20 mg/kg)), group 3 (EESO 200 mg/kg, oral), group

4 (EESO 400 mg/kg, oral), group 5 standard (piracetam 200 mg/kg intraperitoneal).

Each value represents the mean percentage of conditioned (CAR) and unconditioned (UAR) avoidances for each group (6mice)  $\pm$  S.E.

### Inference

The electric shock escape or conditioned avoidance percentage for GROUP-V is 82.00% and the GROUP-IV showed promising results with 70.00  $\pm$  3.54% which is lower compared to the GROUP-V, but it shows that the extracts given are acting on improving the animal memory and remembering capabilities.

### Y-maze spontaneous alternation test

#### Treatment schedule

Animals were divided in 5 groups; in each group 6 animals were used. Subgroup 01 (vehicle control: Acacia suspension 1 ml/kg oral), group 02 (Scopolamine (20 mg/kg)), group 3 (EESO 200 mg/kg, oral), group 4 (EESO 400 mg/kg, oral), group 5 standard (piracetam 200 mg/kg intraperitoneal).

Piracetam and EESO were orally administered for 7 successive days. Dementia was induced on the 7th day by single i.p. injection of scopolamine 1 h after the last dose of test agents. Y-maze behavioral test was performed 1 h after scopolamine injection. Total arm entries (TAE) and spontaneous alternation performance (SAP) were measured during 5-min session and % SAP was calculated.

Data are expressed as mean  $\pm$  S.E.M of 6 animals. Statistical analysis was carried out by non-parametric One-Way ANOVA followed by Dunn's multiple comparisons test.

### Inference

In this test the observation is for mice to enter the Y-maze arms in the given intervals and it was found that the results of group-VI(28.04  $\pm$  1.78) are similar to that of standard drug treated mice group-V(29.00  $\pm$  1.56).

### Measurement of locomotor activity

Locomotor activity was measured 24h after performing water maze test in mice of groups 3 to 5 using photoactometer (INCO, Ambala).

#### Treatment schedule

Animals were divided in 5 groups; in each group 6 animals were used. Subgroup 01 (vehicle control: Acacia suspension 1 ml/kg oral), group 02 (Scopolamine (20 mg/kg)), group 3 (EESO 200 mg/kg, oral), group 4 (EESO 400 mg/kg, oral), group 5 standard (piracetam 200 mg/kg intraperitoneal).

### Inference

In this test Scopolamine induced demented mice observed for their motor activity or movement, the GROUP-VI animals showed results (303.48  $\pm$  1.48) similar to that of control group (302.5  $\pm$  1.26) and similar to that of standard group (312.54  $\pm$  1.43) as there is no big difference in the results.

### BRAIN ACETYLCHOLINESTERASE ACTIVITY

Brain acetylcholinesterase was estimated using the method of Ellman et al. Briefly, 0.4 mL of brain homogenate was added to a test tube containing 2.6 mL of phosphate buffer. 0.1 mL DTNB reagent was added to the above mixture and absorbance was noted at 412 nm. 0.02 mL of acetylcholine iodide solution was added and again absorbance was noted 15 min thereafter. Change in absorbance per min was calculated.

The rate of hydrolysis of substrate was calculated using following formula:

$$R = \text{change in absorbance/min} \times 5.74 \times 10^{-4} / C_0,$$

$$R = \text{rate of hydrolysis of acetylcholine iodide/min/mg tissue,}$$

$$C_0 = \text{weight of tissue homogenate in mg/mL.}$$

### Treatment schedule

Animals were divided in 4 groups; in each group 6 animals were used. Subgroup 01 (vehicle control: Acacia suspension 1 ml/kg oral), group 02 (Scopolamine (20 mg/kg)), group 3 (EESO 200 mg/kg, oral), group 4 (EESO 400 mg/kg, oral), group 5 standard (piracetam 200 mg/kg intraperitoneal).

### Inference

The higher the concentration of cholinesterase the higher is the rested state the treatment of Scopolamine induced mice with EESO showed decrease (41.31  $\pm$  1.58) of cholinesterase enzyme.

### Effect of EESO administration for 7 days in biochemical parameters of animal brain antioxidant status

#### Inference

Anti oxidants plays a major role in the body immune system. The higher the concentration the higher is the tissue regeneration capabilities the ethanolic extracts of *salvia officinalis* showed better results as for the concentration of catalase and DPPH activity.

### Histopathological evaluation

#### Inference

In the histopathological evaluation the brain tissues were collected and observed for tissue damage and regeneration for this the GROUP-I animal samples were taken as standard and compared to others. Group-II tissues shows the tissue damage over the treatment period, where the group-III showing slow recovery of the tissues whereas the group-IV and GROUP-V showed regenerated cells to fully regenerated cells.

### CONCLUSION

The studies carried out on Ethanolic extract of *Salvia officinalis* plant at two different doses (200 mg/kg, 400 mg/kg) showed dose dependent activity.

Alzheimer's disease is progressive neurodegenerative disorder which affects older individuals. It is the most common cause of dementia. It is associated with the presence of senile plaques which are deposition of beta- amyloid protein in the hippocampus area of the brain. Herbal medicine offers several options to modify the progress and symptoms of AD. In conclusion the investigation revealed that the *Salvia officinalis* have significant potential in memory and sensory improving/recovery making it possible to continue the study with possible modifications for betterment of the results, though obtained results may not be higher than the standard marketed formulations, these prepared herbal formulations can be used as a secondary source for the treatment. Further studies can be performed by preparing herbal formulations from the isolated compounds and stabilizing the prepared formulations. India is a country has a rich source of plant life further studies are needed to finding out the medicinal properties of the available plant life.

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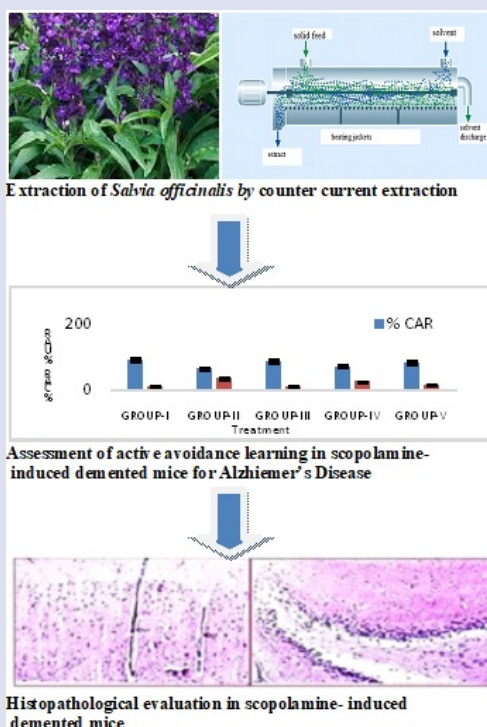
## DECLARATION OF INTEREST

All the authors have no conflicts of interest.

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## GRAPHICAL ABSTRACT



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