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EVALUATION OF ANTI-ALZHEIMER ACTIVITY OF ETHANOLIC EXTRACT OF *ORIGANUM VULGARE* LEAVES IN WISTAR ALBINO RATS

Shivam Varma and Imtiyaz Ansari *

Department of Pharmacology, Oriental College of Pharmacy, Sector 2, Behind Sanpada Railway Station, Sanpada West, Navi Mumbai - 400705, Maharashtra, India.

Keywords:

Alzheimer's Disease, *Origanum vulgare*, Scopolamine, Malonyl Dialdehyde, Acetyl cholinesterase enzyme levels in the brain, Novel object recognition test, Y maze test, Morris water maze

Correspondence to Author:
Mr. Imtiyaz Ansari

Assistant Professor,
Department of Pharmacology,
Oriental College of Pharmacy,
Sanpada, Navi Mumbai - 400705,
Maharashtra, India.

E-mail: imtiyaz.ansari@ocp.edu.in

ABSTRACT: Alzheimer's disease, the most frequent cause of dementia, is a major global health concern with enormous societal and individual ramifications. Some of the hallmarks linked with AD include a decrease in acetylcholine (ACh) levels in the synapse, the buildup of extracellular beta-amyloid plaques, and tau protein intraneuronal tangles. Low levels of ACh have been linked to memory and reasoning problems. Plaques may disrupt signal transmission from neuron to neuron, resulting in cell death, whereas tau tangles obstruct the passage of vitamins and other essential nutrients within neurons. The efficacy of an ethanolic extract of *Origanum vulgare* leaves (OVE) against scopolamine-induced Alzheimer's disease in Wistar albino rats was investigated in this work. OVE was given to rats at doses of 100 mg/kg body weight, 200 mg/kg body weight, and 300 mg/kg body weight at a dose of 2.5 mg/kg. Morris water maze, Novel object recognition test and Y-maze were used to examine anti-Alzheimer activity, as well as biochemical tests such as neurotransmitter Acetyl cholinesterase activity, catalase activity, malonyl dialdehyde and Nitric oxide assays. The results showed that extract administration inhibits Alzheimer's disease and boosts acetylcholine and catalase levels, while phytochemical tests revealed the presence of Phenolic chemicals, flavonoids and alkaloids in the OVE. As a result, *Origanum vulgare* leaves extract appears to be a promising candidate for improving learning and memory, as well as for treating amnesia and Alzheimer's disease.

INTRODUCTION: Dr. Alois Alzheimer, a German scientist, was the first to report Alzheimer's disease in 1907. He called it a 'neurodegenerative disease,' which means that the neural system is always degenerating. It was marked by a steady deterioration in cognitive abilities as well as severe behavioral abnormalities such as restlessness, irritability, confusion, melancholy, and anxiety.

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It was later defined as "a progressive neurodegenerative condition characterized by loss of memory, confusion, intellectual disability, social retreat, poor judgement and other symptoms caused by irreversible death of neurons within the cerebral cortex and hippocampus area [1]. The most common type of dementia is Alzheimer's disease.

It affects more than 50 million people globally, with the number expected to climb to 152 million by 2050 if no cure or preventive measures are found [2]. The prevalence of Alzheimer's disease rapidly increases from roughly 2-3 percent in adults aged 70-75 years to 20–25 percent in those aged 85 years or more. There isn't enough data to say whether the prevalence of Alzheimer's disease is increasing or decreasing.

Ladies are more prone than males to develop Alzheimer's disease in later life, owing to an age-related rise in the risk of the disease. A few studies have found that the general prevalence of Alzheimer's disease varies greatly between countries and is influenced by social and economic factors [3].

Some hallmarks associated with AD include a decrease in acetylcholine (ACh) levels in the synapse [4] accumulation of extracellular beta-amyloid plaques [5] and tau protein intraneuronal tangles [6]. Low levels of ACh have been linked to memory and reasoning problems. Plaques can disrupt signal transmission from neuron to neuron, resulting in cell death, whereas tau tangles can obstruct the delivery of vitamins and other essential nutrients within neurons [7]. When the early symptoms of Alzheimer's disease appear, the brain can adjust and the sick person can operate normally. As nerve cells are injured, the cerebrum is unable to compensate for the changes, and the individual's intellectual capacity gradually declines. Plaques and tangles occur not only in certain areas of the brain associated with perceived capacity, but also in other portions of the brain throughout time. After then, nerve cell damage is severe enough that people experience an obvious intellectual decline, such as memory loss or disorientation in place or time, as well as social side effects such as melancholy, character changes and a loss of interest for activities they used to enjoy. Fundamental actual capacities, such as swallowing, are hampered much later [8].

Current Treatment of Alzheimer's disease in Medical Science: The existing drug therapy mainly targets the symptoms of AD, such as cognitive impairments, rather than alsohalting or preventing the causal neuropathology. The only approved drugs for the treatment of AD by many national regulatory authorities, including the Food and Drug Administration in the United States, are acetylcholinesterase (AChE) inhibitors (AChE-Is) (*e.g.,* donepezil, rivastigmine, galantamine and tacrine) and memantine, which is an NMDA antagonist.

Origanum vulgare Linn: *Origanum vulgare* (Lamiaceace), sometimes known as oregano, is an herbaceous perennial native to Europe, North Africa and temperate Asian countries. Origanum species can be found in abundance in mountainous and hilly places with a wide variety of altitudes. Oregano is a mint-family flowering plant (Lamiaceace). It is found throughout temperate Western and Southwestern Eurasia, as well as the Mediterranean. Oregano is a perennial herb that grows 20–80 cm tall and has alternating leaves that are 1–4 cm long. Purple flowers are formed in tall spikes and are long and purple [9]. *Origanum vulgare* is used by tribal peoples in the western Himalayan belt to treat flatulence, diaphoresis and cough, as well as to stimulate menstrual discharge, energies the body, enhance hunger, and as a tonic [10]. The antioxidant and antibacterial properties of oregano have been studied, with a focus on food preservation. Thymol, carvacrol, g-terpinene, p-cymene, linalool, terpinen-4-ol and sesquiterpenoids are the main components found in this plant. In medicine and the food business, essential oils of Origanum species containing mostly carvacrol and/or thymol, as well as significant levels of g-terpinene and p-cymene, have been employed [11].

MATERIALS AND METHODS:

Plant Material: *Origanum vulgare* leaves were obtained in November 2021 from leanbeing healthcare New Delhi and were authenticated by Mr. Mahesh Atale, M.Sc. Botany, Alarsin-Pioneers in Ayurvedic Research, Mumbai- 400 093 and specimen were submitted to Pharmacology department, Oriental College of Pharmacy, Sanpada, Navi Mumbai - 400705.

Preparation of Extract: *Origanum vulgare* leaves will be collected and dried in shadow at room temperature; the leaves will be powdered and soaked in ethanol 70% at room temperature for 48 hours. During this time, the mixture will be stirred intermittently finally the prepared solution will be filtered using filter paper and kept in Bain Marie at 40°C for 72 h to obtain dried powder.

Medicinal Properties: Anti-oxidant activity, Antiurolithic activity, Anti-hyperglycaemic activity, Antithrombin activity, Antibacterial and anticancer activity, Wound healing activity, Antifungal activity, Hepatoprotective activity, Anti-hyperlipidemic actions, Anti-obesity activity.

Toxicity: The alcoholic extract's LD_{50} was estimated to be 2000 mg/kg. This plant has also known to be edible making it safe and accordingly, $1/10^{th}$ and $1/20^{th}$ dose were selected for study *i.e.*, low dose 100 mg/kg, intermediate dose 200 mg/kg and high dose 300 mg/kg for the test groups with dose conversion.

Drug Treatment: The ethanolic extract was suspended in Normal saline in doses of 100, 200,300 mg/kg p.o. for the pharmacological tests. The doses were selected based on the previous studies on the ethanolic extract. *Origanum vulgare* leaves extract (OVE) was administered to individual rats in group 4,5,6,7. None of the rat was dead due to treatment till the end of the observation period. The *Origanum vulgare* leaves extract (OVE) caused no abnormality till the duration of treatment.

Qualitative Phytochemical Screening: Preliminary chemical studies were performed on *Origanum vulgare* alcoholic extract. The presence of following phytoconstituents were assessed: Molisch test, Resorcinol test, were done for carbohydrates, Lead acetate test, Ferric Chloride ($FeCl_3$) test, Conc. Sulphuric acid test for flavonoids, Modified Borntrager's test, Aqueous Sodium Hydroxide (NaOH) test for glycosides, Ferric chloride test and dil. Iodine solution test for tannins, Liebermann Burchard test for terpenoids, Iodine test, Ferric Chloride ($FeCl_3$) test, Lead Acetate test, Potassium dichromate test and Ellagic acid test were done for phenoliccompounds.

Equipments and Chemicals: Electronic balance, Morris water maze, Y-maze, novel object apparatus, syringes, and needles, ethanol, NS, and scopolamine. Donepezil tablet, 5, 5'-dithiobis-(2-nitrobenzoic acid) (DTNB), acetylcholine, thiobarbituric acid (TBA), Trichloroacetic acid (TCA), Hydrogen peroxide (H_2O_2), Griess reagent.

Animals: The CPCSEA registered Institutional Animal Ethics Committee (IAEC) of Oriental College of Pharmacy; Sanpada approved the animals needed for the research work with protocol no. OCP/IAEC/2020-2021/10 entitled "Evaluation of anti-Alzheimer activity of ethanolic extract of *Origanum vulgare* leaves in Wistar albino rats.]". Animals procured were female Swiss Albino mice (15 to 20 g) for carrying out acute oral toxicity study & both male and female Albino Wistar rats (100 to 120 g) were procured from National Institute of Biosciences, Pune. The animals were kept in well ventilated, air-conditioned animal house at a constant temperature of 24 ± 2 °C, with 12:12 hours dark: light cycle and with a relative humidity of 55-60%. The animals were placed on bedding material, in spacious polypropylene cages with a paddy husk. The animals were held on normal diet with pellets and filtered water.

Acute Oral Toxicity Study: Acute oral toxicity analysis was carried out for the ethanolic extract of dried Leaves of *Origanum vulgare* as recommended in OECD Guideline 423. The animals were noted keenly for the display of any toxic signs or symptoms at different time intervals of 0, 30 min, 1, 2, 4, 6, 8, 12 h and then daily for a period of 14 days. When this was conducted, any kind of toxic signs were not noted in clinical parameters during acute toxicity study even at the highest dose of 2000mg/kg. Hence, it can be concluded that the LD_{50} of the ethanolic extract of dried Leaves of *Origanum vulgare* is greater than 2000 mg/kg.

Experimental Design: Grouping of Animals: Animals were divided into seven groups, each of six animals.

- Group I: Control group oral administered by Distilled water (20 ml/kg)
- Group II: Intraperitoneal injection by scopolamine hydrochloride (0.5 mg/kg) (Disease control)
- Group III: Animal oral administered by donepezil tablet standard (2.5 mg/kg) and Alzheimer's induced with scopolamine (Standard)
- Group IV: Animal's oral administered by extract which is dissolved in NS (100 mg/kg) and Alzheimer's induced with scopolamine (Low dose)
- Group V: Animal's oral administered by extract which is dissolved in NS (200 mg/kg) and Alzheimer's induced with scopolamine (Intermediate dose)

- Group VI: Animal's oral administered by extract which is dissolved in NS (300 mg/kg) and Alzheimer's induced with scopolamine (High dose)
- Group VII: Animal's oral administered by extract which is dissolved in NS (300 mg/kg) (Extract only)

Anti-Alzheimer Study:

Morris Water Maze: The apparatus consists of a circular pool with a featureless inner surface (45 cm in height and 100 cm in circumference). The pool was filled to a height of 30 cm with opaque water (kept at 22 2°C) and divided into four quadrants of equal area, each indicated by a different visual cue. At the centre of one of the four quadrants, a platform (29 cm x 6 cm) was placed one centimeter below the water level (the target quadrant). The platform remained in the same position throughout the length of the experiment. The test began on the tenth day after therapy, when the rats were permitted to swim for 120 seconds without the platform to acclimate.

Each animal received four 120-second learning trials with a 60-second intertrial interval over the next four days. The rat was placed in the water facing the pool wall diagonally opposite the quadrant where the platform was kept for each learning experiment. For each experiment, the time it took the animal to locate the submerged platform was recorded as the escape latency time. When the animal was unable to identify the platform within 120 seconds, it was led to the platform and permitted to rest there for 60 seconds, resulting in a 120-second escape latency time. The concealed platform trials or acquisition test were recorded during these sessions. The platform was removed from the water on day 15 (24 hours after the final learning trial) and the rats were given a probe experiment to test memory retention. Each rat was dropped into the water diagonally opposite the target quadrant and given 60 seconds to swim back to the quadrant where the platform had been placed before. The amount of time the animal spent in the target quadrant was tracked [12].

Novel Object Recognition Test: A habituation phase an acquisition phase, and a retention phase are the three steps of the procedure. Rats were individually subjected to a 10-minute adaption session during which they were introduced to the unfilled area in order to become accustomed with the apparatus on the first day (habituation Phase). The animals were treated to a 10 min session on the second day (acquisition phase) during which floor-fixed two objects (A and B) were put in a symmetric position in the centre line of the area. The two objects were distinct in shape but identical in size, having been made from the same material and having a similar colour and fragrance. The rats were free to investigate the things in the open area. To demonstrate the exploring activities of rats, the exploration time on each object was displayed (in seconds). Rats were allowed to explore the open field on the third day (retention phase) in the presence of two objects: a recognizable object A and a novel object C of different shapes but similar colour and size (A and C). For each rat, the recognition index (for retention session) was determined as the ratio recognition index (RI) = Time exploring novel item/ (Time exploring novel object + Time exploring familiar object) *100 [13].

Y Maze Test: The Y maze was made up of three arms, each 40 cm long, 12 cm tall, 3 cm broad at the bottom and 10 cm wide at the top and converged in an equilateral triangular central space. During an 8-minute session, each rat was attached to one arm and permitted to freely navigate the maze. To be able to alternate, the rat must be aware of which arm they have already visited.

The sequences of arm entrances were visually recorded, including possible returns into the same arm. The performance of instant working memory was measured by observing spontaneous alternation behaviour. When the rat's hind paws had completely entered the arm, it was termed complete entry. On overlapping triplet sets, alternation was defined as sequential entries into the three separate arms (A, B, and C). The formula was used to compute the percent change. % Alteration = (Number of arm alterations/total arm entry-2) *100 [14].

Biochemical Test:

Preparation of Brain Sample: Following the evaluation of learning and memory paradigms in scopolamine-induced amnesia, rats from each group were euthanized using a carbon dioxide

chamber; brains were rapidly removed and placed in ice cold saline. On a petri plate chilled on ice cubes, the frontal cortex, hippocampus and septum (or any other region of interest) were swiftly torn out (this part of the brain cannot be identified in small rat brain therefore whole brain was taken). In 0.1M Phosphate buffer, the tissues were weighed and homogenized (pH 8). The researchers took samples of rat brain homogenates and examined them for acetylcholine esterase, catalase, glutathione and MDA, among other things. Enzymatic tests were performed on the supernatant.

Estimation of Acetyl Cholinesterase Enzyme Levels in the Brain: A 0.4 ml portion of the homogenate was put to a cuvette with 2.6 ml phosphate buffer (0.1M, pH 8) and 100l DTNB. The contents of the cuvette were completely mixed with bubbling air and the absorbance was measured in a spectrophotometer at 412 nm. 20 l of acetyl thiocholine, the substrate, was added and the change in absorbance was measured. As a result, the change in absorbance per minute was calculated [15].

Determination of Catalase (Cat) Activity: In a cuvette containing 1.9 ml of 50 mM phosphate buffer, 0.1 ml of supernatant was added (pH 7.0). The reaction was initiated by adding 1.0 mL of freshly produced 30 mM H_2O_2 to the mixture. Changes in absorbance at 240 nm were used to evaluate the rates of H_2O_2 breakdown spectrophotometrically. Catalase activity was measured in units per milligram of protein. At a wavelength of 240 nm, the absorbance was measured [16].

Determination of Nitric Oxide (NO): The accumulation of nitrate in the supernatant, which was evaluated using a colorimetric assay with the Griess reagent, was used to assess nitric oxide generation (1:1 solution of 1 percent sulfanilamide in 5 percent H_3PO_4 and 1 percent naphthylamine diamine dihydrochloric acid in water).

The supernatant and Griess reagent were mixed in an equal volume and incubated at room temperature for 10 min in the dark. A spectrophotometer was used to measure the absorbance at 540 nm. A sodium nitrite standard curve was used to estimate the concentration of nitrite in the supernatant [17].

Determination of Malonyldialdehyde (MDA): In a tube, suspension media was extracted from tissue homogenate. It was then treated with 0.5 mL of Trichloroacetic acid (TCA) and 0.5 mL of 8 percent Thiobarbituric acid (TBA) reagent.

The tubes were wrapped in aluminium foil and placed in an 80°C water bath for 30 minutes. After 30 minutes, the tubes were removed and placed in cold water for another 30 min. These tubes were centrifuged at 3000 rpm for 15 min.

At room temperature, the absorbance of the supernatant was measured at 540 nm against a blank solution. The MDA value was calculated as moles MDA/mg protein [17].

Statistical Analysis: The data were analyzed using BioStat Pro (version 5.9.8). For each category the results are expressed as mean ± SEM. A one-way variance analysis (ANOVA) was used to analyze statistical differences followed by the Tukey-Kramer test.

Tests at $P \leq 0.05$. were considered statistically significant. *** indicated $p \leq 0.001$, ** indicates $p \leq 0.01$, * Indicates $p \leq 0.1$

RESULTS:

Qualitative Phytochemical Screening:

TABLE 1: RESULT OF PHYTOCHEMICAL ANALYSIS OF ALCOHOLIC EXTRACT OF THE DRIED LEAVES OF *ORIGANUM VULGARE*

S. no.	Phytochemicals	Tests performed	Inference	Alcoholic extract of the dried leaves of *Origanum vulgare*
1	Carbohydrates	Molisch test	Violet ring at junction	+
		Benedict test	Red ppt	+
		Selwinoff test	Red colour	+
2	Flavonoids	Sodium hydroxide (NaOH) test	Yellow ppt	+
		Shinoda test	Pink to red solution	+

		Alkaline reagent test	Colourless	+
3	Glycosides	Modified Borntrager's test	Pink to red colour	+
4	Tannins	Ferric chloride test	Deep black colour	+
		dil. Iodine solution test	Transient red colour	+
5	Saponins	Foam test	Persistent foam	-
6	Fixed oils	Sodium hydroxide solution test	Clear blue solution	+
7	Alkaloids	Dragendroff reagent test	Reddish brown ppt	-
		Mayer's reagent test	Buff coloured ppt	-
		Hager's reagent test	Yellow ppt	-
8	Phenols	dil. Potassium permanganate test	Decolouration of KMnO4 solution	+
9	Sterols	Liebermann Burchard test	Green upper layer	+
		Salkowski test	Red lower layer	+
10	Terpenoids	Liebermann Burchard test	Red upper layer	+
11	Proteins	Biuret test	Violet/ pink colour	-

Present (+) /Absent (-)

Acute Oral Toxicity Studies: Acute oral toxicity analysis was carried out for the ethanolic extract of dried Leaves of *Origanum vulgare* as recommended in OECD Guideline 423.

The animals were noted keenly for the display of any toxic signs or symptoms at different time intervals of 0, 30 min, 1, 2, 4, 6, 8, 12 h and then daily for a period of 14 days. When this was conducted, any kind of toxic signs were not noted in clinical parameters during acute toxicity study even at the highest dose of 2000mg/kg. Hence, it can be concluded that the LD_{50} of the ethanolic extract of dried Leaves of *Origanum vulgare* is greater than 2000 mg/kg.

Anti-Alzheimer Study:

Morris Water Maze: Scopolamine treatment resulted in considerably higher escape latencies during the acquisition sessions (days 11–14), although this effect was minimised by co-administration of the test formulation. In the control group, significant reductions in escape latencies were observed on days 2–4 (treatment days 12–14) of the acquisition sessions when compared to day 1 (treatment day 11) of the acquisition sessions, but not in the scopolamine-treated rats. In rats given both scopolamine and the test formulation, these differences were recovered. On day 15, the scopolamine-treated rats spent considerably less time swimming in the target quadrant than the controls, whereas the rats co-administered with the test formulation and scopolamine spent significantly more time in the target quadrant than the disease controls.

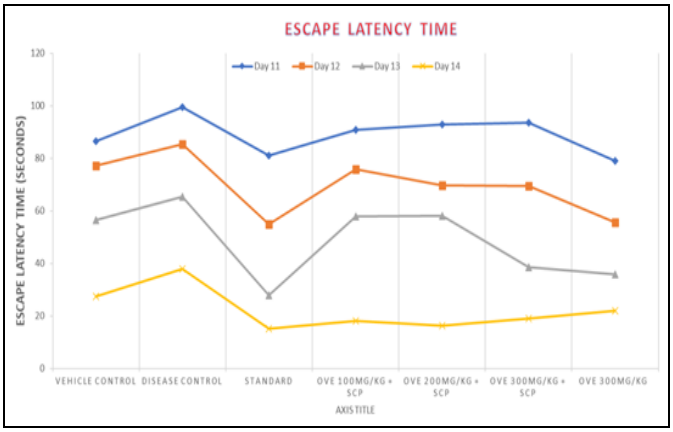

FIG. 1: EFFECT OF ETHANOLIC EXTRACT OF *ORIGANUM VULGARE* LEAVES ON ESCAPE LATENCY TIME IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RAT

TABLE 2: EFFECT OF ETHANOLIC EXTRACT OF *ORIGANUM VULGARE* LEAVES ON ESCAPE LATENCY TIME IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS

Day Group	Escape Latency Time (Seconds)			
	Day 11	Day 11	Day 11	Day 11
Control	86.458±4.0412	77.208±0.6272	56.625±0.8915	27.3333±0.9212
Disease Control	99.5±2.698#	85.375±0.9827#	65.375±0.826#	37.9583±1.0595#
Standard Drug	81.083±3.2357***	55±0.7528***	27.9167±0.7207***	15.2083±1.2373***
Lowdose (100 mg/kg)	90.833±1.1322***	75.875±1.0777***	57.833±1.3899***	18.208±0.6239***
Medium Dose (200 mg/kg)	92.833±0.7379***	69.125±0.9677***	58.042±1.2356***	16.417±0.7435***
High Dose (300 mg/kg)	93.5±0.9895***	69.583±0.5465***	38.625±1.5203	19.083±0.5028***
Extractiononly (300 mg/kg)	79±1.812***	55.667±1.734***	35.833±1.8333	21.958±0.6905***

All the four-test groups of ethanolic extract of leaves *Origanum vulgare* showed dose dependent increase in Escape latency time when compare against negative control group (scopolamine).

TABLE 3: EFFECT OF ETHANOLIC EXTRACT OF *ORIGANUM VULGARE* LEAVES ON TIME SPENT IN TARGET QUADRANT IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS

Groups	Recognition Index
Control	12.833±0.600
Disease Control	7.166±0.749
Standard Drug	22±1.211
Low Dose (100mg/kg)	13.333±0.557
Medium Dose (150mg/kg)	14.167±0.703
High Dose (250mg/kg)	17.833±0.477
Extract Only (250mg/kg)	19.833±1.013

Values are the mean ± SEM of n=6 rats/treatment. Significance ***p ≤0.001.

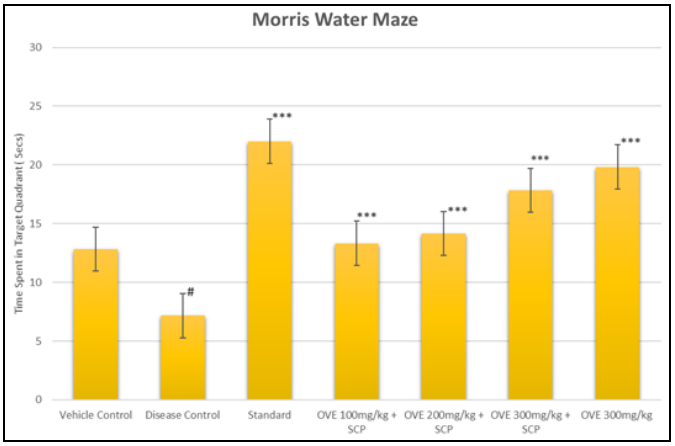

FIG. 2: EFFECT OF ETHANOLIC EXTRACT OF *ORIGANUM VULGARE* LEAVES ON TIME SPENT IN TARGET QUADRANT IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RAT

Novel Object Recognition Test: All the four-test groups of ethanolic extract of leaves *Origanum vulgare* showed dose dependent increase in recognition index when compare against negative control group (scopolamine).

TABLE 4: EFFECT OF ETHANOLIC EXTRACT OF *ORIGANUM VULGARE* LEAVES ON RECOGNITION INDEX IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS

Groups	Recognition Index
Control	Recognition Index
Disease Control	55.68±1.062
Standard Drug	42.16±1.3062
Low Dose (100 mg/kg)	60.743±1.273
Medium Dose (200 mg/kg)	55.96±1.020
High Dose (300 mg/kg)	56.879±1.295
Extract Only (300 mg/kg)	59.66±0.862

Values are the mean ± SEM of n=6 rats/treatment. Significance ***p ≤0.001.

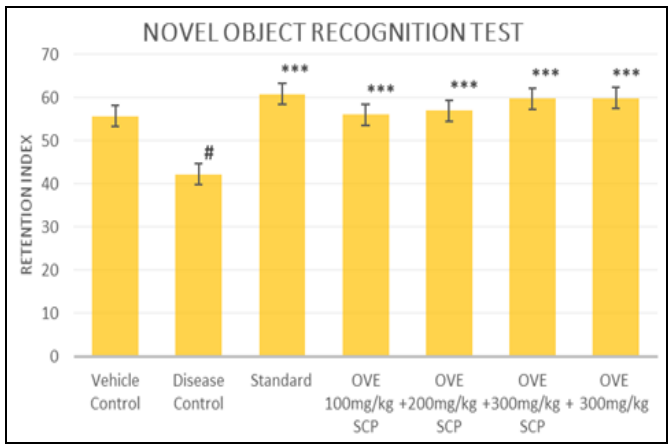

FIG. 3: EFFECT OF ETHANOLIC EXTRACT OF *ORIGANUM VULGARE*LEAVES ON RECOGNITION INDEX IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RAT

Y-Maze: All the four-test groups of ethanolic extract of leaves *Origanum vulgare* showed dose dependent increase in percentage alteration when compare against negative control group (scopolamine).

TABLE 5: EFFECT OF ETHANOLIC EXTRACT OF *ORIGANUM VULGARE* LEAVES ON % ALTERATION IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS

Groups	Percentage Alteration
Control	54.29±0.803
Disease Control	32.28±0.609
Standard Drug	57.21±0.542
Low Dose (100mg/kg)	45.42±0.181
Medium Dose (150mg/kg)	47.83±0.685
High Dose (250mg/kg)	53.53±1.141
Extract Only (250mg/kg)	55.81±0.978

Values are the mean ± SEM of n=6 rats/treatment. Significance ***p ≤0.001.

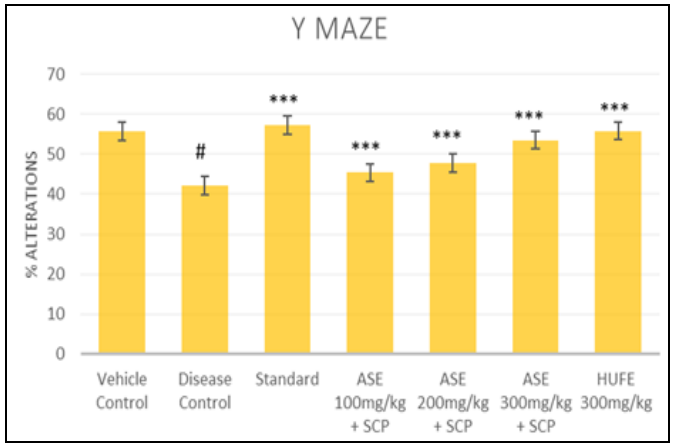

FIG. 4: EFFECT OF ETHANOLIC EXTRACT OF *ORIGANUM VULGARELEAVES* ON % ALTERATION IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RAT

Biochemical Test:

Estimation of Acetyl Cholinesterase Enzyme Levels in the Brain: All the four test doses of the ethanolic extract of leaves of *Origanum vulgare* showed dose dependent decrease in acetyl cholinesterase level when it was compared against negative control group (scopolamine).

TABLE 6: EFFECT OF ETHANOLIC EXTRACT OF *ORIGANUM VULGARE* LEAVES ON ACETYLCHOLINE ESTERASE LEVEL IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS

Groups	Enzyme Level
Control	0.081±0.0003
Disease Control	0.092±0.0003
Standard Drug	0.071±0.0003
Low Dose (100 mg/kg)	0.078±0.0015
Medium Dose (200 mg/kg)	0.079±0.00013
High Dose (300 mg/kg)	0.075±0.0001
Extract Only (300 mg/kg)	0.074±0.0015

Values are the mean ± SEM of n=6 rats/treatment. Significance ***p ≤0.001.

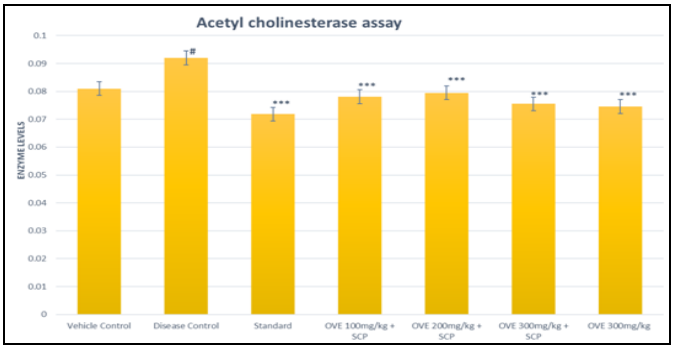

FIG. 5: EFFECT OF ETHANOLIC EXTRACT OF *ORIGANUM VULGARE* LEAVES ON ACETYLCHOLINE ESTERASE IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RAT

Determination of Catalase (Cat) Activity: All the four test doses of the ethanolic extract of leaves of *Origanum vulgare* showed dose dependent increase in catalase activity when it was compared against negative control group (scopolamine).

TABLE 7: EFFECT OF ETHANOLIC EXTRACT OF *ORIGANUM VULGARE* LEAVES ON CATALASE ACTIVITY IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS

Groups	Catalase Level
Control	34.67±0.120
Disease Control	27.38±0.116
Standard Drug	39.39±0.154
Low Dose (100 mg/kg)	33.62±0.433
Medium Dose (200 mg/kg)	35.41±0.589
High Dose (300 mg/kg)	37.48±0.513
Extract Only (300 mg/kg)	38.17±0.559

Values are the mean ± SEM of n=6 rats/treatment. Significance ***p ≤0.001.

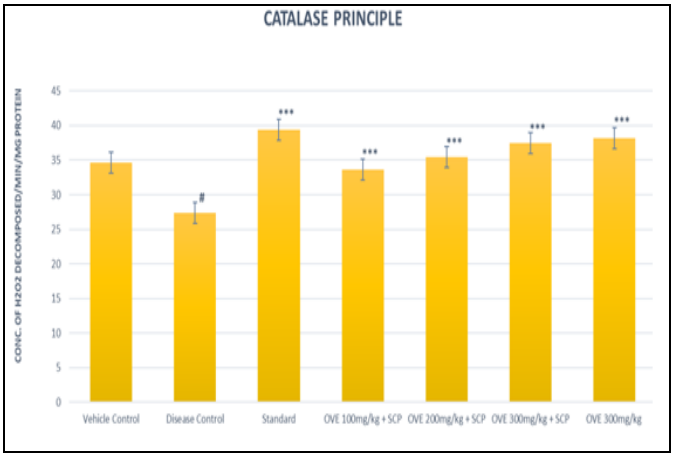

FIG. 6: EFFECT OF ETHANOLIC EXTRACT OF *ORIGANUM VULGARE* LEAVES ON CATALASE LEVELS IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RAT

Determination of Nitric Oxide (NO): All the four test doses of the ethanolic extract of leaves of *Origanum vulgare* showed dose dependent increase in nitric oxide activity when it was compared against negative control group (scopolamine).

TABLE 8: EFFECT OF ETHANOLIC EXTRACT OF *ORIGANUM VULGARE* LEAVES ON NITRIC OXIDE ACTIVITY IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS

Groups	NO Level
Control	7.06±0.021
Disease Control	20.0475±0.243
Standard Drug	9.14±0.104
Low Dose (100 mg/kg)	14.90±0.128
Medium Dose (200 mg/kg)	13.36±0.197
High Dose (300 mg/kg)	11.94±0.086
Extract Only (300 mg/kg)	10.41±0.140

Values are the mean ± SEM of n=6 rats/treatment. Significance ***p ≤0.001

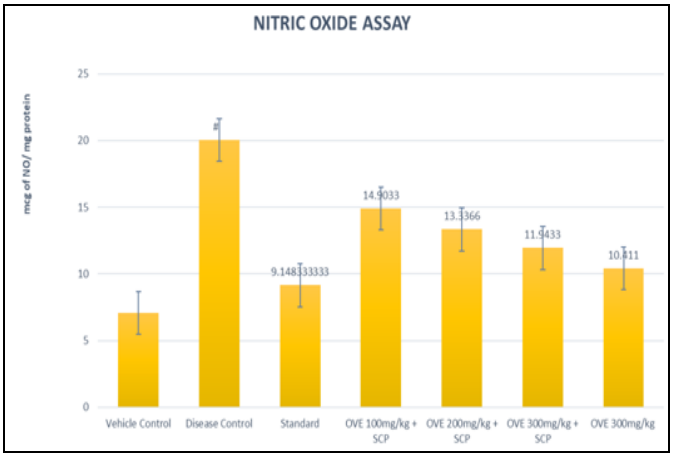

FIG. 7: EFFECT OF ETHANOLIC EXTRACT OF *ORIGANUM VULGARE* LEAVES ON GLUTATHIONE PEROXIDES LEVEL IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS

Determination of Malonyldialdehyde (MDA): All the four test doses of the ethanolic extract of leaves of *Origanum vulgare* showed dose dependent decrease in MDA level activity when it was compared against negative control group (scopolamine).

TABLE 9: EFFECT OF ETHANOLIC EXTRACT OF *ORIGANUM VULGARE*LEAVES ON MDA LEVEL IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS

Groups	Mda LEVEL
Control	0.025±0.001
Disease Control	0.030±0.0003
Standard Drug	0.0103±0.0004
Low Dose (100 Mg/Kg)	0.0206±0.0008
Medium Dose (200 Mg/Kg)	0.0201±0.0006
High Dose (300 Mg/Kg)	0.0166±0.0006
Extract Only (300 Mg/Kg)	0.0141±0.0007

Values are the mean ± SEM of n=6 rats/treatment. Significance ***p ≤0.001.

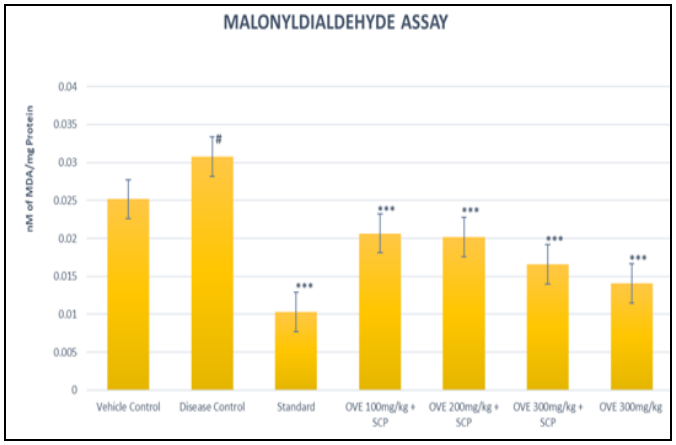

FIG. 8: EFFECT OF ETHANOLIC EXTRACT OF *ORIGANUM VULGARE* LEAVES ON MDA LEVEL IN SCOPOLAMINE INDUCED ALZHEIMER IN ALBINO WISTAR RATS

DISCUSSION: Alzheimer's disease (AD) is a degenerative brain illness characterized by persistent higher neurological abnormalities of brain activity and functions. It implies that there are barriers in the areas of memory, judgement, visual identification, emotions and so on.

The prevalence of Alzheimer's disease and related dementias is rising as the population ages. Because the loss of cholinergic neurons in the brain is connected to cognitive decline, a decrease in cholinergic neurons in the brain of Alzheimer's patients leads to cognitive impairment and memory loss. Senile plaque and neurofibrillary tangle formation, oxidative and inflammatory processes and neurotransmitter abnormalities are pathogenic characteristics of AD in the central nervous system (CNS). In the Morris Water Maze test, leaves of *Origanum vulgare* were found to have anti-Alzheimer properties.

The MWM task has long been used to assess rodent spatial learning and memory. In a dose-dependent way, it exhibited a decrease in escape latency time and an increase in time spent in the target quadrant. Y-maze task is an efficient spatial memory test. Y maze showed increase in % alteration.

The Novel Object Recognition experiment is used to measure memory, particularly memory recognition, in animal models of neurological diseases. In a test of novel object recognition, the recognition index increased. It shows that rats' spatial memory has improved. Direct study of neurotransmitter content in the cerebral cortex of Alzheimer's patients reveals a decrease in the level of Ach.

GSH is a prominent endogenous enzyme that catalyses antioxidants and plays a key role in the detoxification of reactive oxygen species in AD. Catalase is also a common enzyme found in almost every living organism. It catalyses hydrogen peroxide breakdown into water and oxygen. Alkaloid, phenol, flavonoid and steroid constituents in the plant lower acetylcholinesterase activity while increasing catalase and GSH activity, resulting in antioxidant activity.

Reactive oxygen species are to blame for the age-related decline in cognitive ability, which may eventually lead to Alzheimer's disease in the elderly. *Origanum vulgare* has also been shown to have antioxidant properties.

The neuroprotective effect of *Origanum vulgare* leaves extract may be related to its antioxidant properties, which expose vulnerable brain cells to less oxidative stress, minimizing brain damage.

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